Vol. XII, No. 3 July, 1925

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

PUBLICATIONS COMMITTEE

W. L. BALLS
V. H. BLACKMAN
A. D. IMMS
R. T. LEIPER
G. A. K. MARSHALL

CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

PRINTED IN GREAT BRITAIN

The Association of Economic Biologists

President

PROF. V. H. BLACKMAN, Sc.D., F.R.S.

Vice-Presidents

E. J. BUTLER, D.Sc., C.I.E., M.B.
A. D. IMMS, D.Sc.

Hon. Treasurer

A. D. IMMS, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Editors

W. B. BRIERLEY, D.Sc.
D. WARD CUTLER, M.A.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (General and Botanical)

S. G. PAINE, D.Sc.,
Botany Department,
Imperial College of Science,
London, S.W. 7

Hon. Secretary (Zoology)

J. WATERSTON, M.A., D.Sc.,
Nat. Hist. Museum,
South Kensington, S.W. 7

Council

PROF. J. H. ASHWORTH, F.R.S.
A. W. BORTHWICK, D.Sc.
E. J. BUTLER, D.Sc., C.I.E., M.B.
D. M. CAYLEY, D.Sc.
A. D. COTTON, F.L.S.
J. C. F. FRYER, M.A.
T. GOODEY, D.Sc.
WM LOBJOIT
G. H. PETHYBRIDGE, D.Sc.
PROF. E. B. POULTON, M.A., D.Sc., LL.D., F.R.S.
LT.-COL. SIR DAVID PRAIN, C.M.G., C.I.E., LL.D., F.R.S.
E. S. SALMON, F.L.S.

CONTENTS OF VOL. XII, No. 3

PAGE

1. The Anatomy of the Leaf of a New Fibre Plant. By R. H. DENNISTON. (With 9 Text-figures) 307
2. Some Infection Experiments with Loose and Covered Smuts of Oats which Indicate the Existence in them of Biological Species. By KATHLEEN SAMPSON, M.Sc. (Lond.). With 2 Text-figures) 314
3. Note on the Bacterial Soft Rot of Turnips. By H. WORMALD and R. V. HARRIS . . . 326
4. A Physiological Study of the Common Bacteria of Air, Soil and Water with a View to their Exact Diagnosis. By MANECK M. MEHTA 330
5. On the Structure, Life-History, Economic Importance and Distribution of the Cocksfoot Moth, *Glyphipteryx fischeriella*, Zell. By ROSHAN LAL CHOPRA, M.A. (Punjab), Ph.D. (Wales). (With 21 Text-figures and 4 Diagrams) 359
6. A Preliminary Note on the Perithecia of *Nectria galligena*. By W. A. R. DILLON WESTON, B.A. (With 2 Text-figures) 398
7. Review 401

VOLUME XII JULY, 1925 No. 3

THE ANATOMY OF THE LEAF OF A NEW FIBRE PLANT

BY R. H. DENNISTON.
Professor of Botany, University of Wisconsin, Madison.

(With 9 Text-figures.)

THE long, slender leaves of *Neoglaziovia variegata* Mez, a Brazilian bromeliaceous species, are of interest because of their fibre content. These leaves are upward of six feet in length, and one to two inches broad at the base. They taper gradually to a point, the margins being armed with sharp spines about one inch apart (Fig. 1).

Fig. 1. Tip of leaf. (Natural size.)

The leaf in cross-section is flattened on one side and convex on the other, the thickest part being at the middle and tapering abruptly to a thin margin.

The cross-section (Fig. 2) shows two distinct regions, an abaxial portion in which the fibro-vascular bundles and bundles of fibres are located, and an adaxial region made up of water-storing parenchyma.

The epidermis, in cross-section (Fig. 8), consists of a single layer of short, tangentially elongated cells protected at the outside by a heavy cuticle. Beneath the epidermis are two to three rows of thick-walled, prosenchymatous cells rounded in cross-section showing numerous simple pits (Fig. 9). They form a continuous sheet except within the stomata.

The rounded, abaxial side of the leaf shows a series of narrow longitudinal ridges. The stomata are of the depressed type located between the ridges. Beneath each stoma is a respiration cavity surrounded by parenchyma cells (Fig. 8). In cross-section the ridges of the leaf appear as a series of rounded elevations each one containing about twenty epidermal cells in a curved line over a group

of subepidermal cells and parenchyma, often including also a small group of fibres.

The parenchyma of the outer (abaxial) region of the leaf is composed of rounded, more or less isodiametric cells containing a few chloroplasts, which often enclose starch grains. In this region are groups of bast fibres which are arranged in radial rows.

At the inner portion of this region are the fibro-vascular bundles (Figs. 3 and 4). These are of the collateral type, with crescent-shaped groups of mechanical cells at the outer and inner sides. Four large vessels with spiral and pitted walls are usually present. The phloem makes up a triangular group of cells on the abaxial side of the xylem. The cell

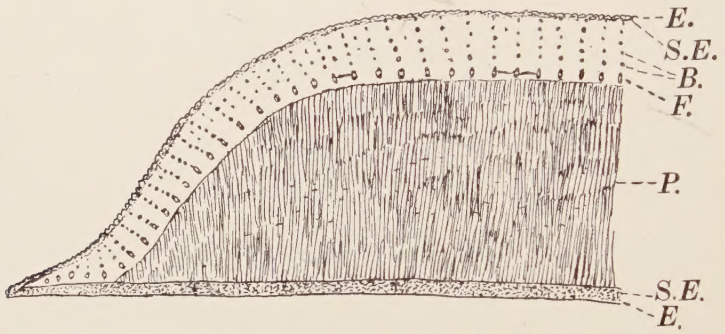


Fig. 2. Diagram of transverse section of portion of leaf. ($\times 6\frac{1}{2}$.)

walls are thin and composed of cellulose. Occasionally two or more bundles are connected by lateral extensions (Fig. 2).

The adaxial portion of the leaf is composed of radially elongated parenchyma cells, whose function is water storage. The adaxial surface of the leaf is not in ridges, and stomata are less frequent than on the abaxial surface.

Description of fibres.

The bast fibres in the parenchyma are in rounded groups (Fig. 5); the larger groups being near the fibro-vascular bundles and the smaller ones toward the outside of the leaf.

The fibres are closely packed and angular with somewhat rounded corners. The walls are thick and the lumen is rounded or flattened or sometimes almost obliterated. The lumen is not always at the centre, indicating that the walls are not uniformly thickened. When macerated in sodium carbonate solution and teased apart, they appear long and

narrow with tapering pointed ends (Fig. 6). The diameter of the lumen varies in different parts of the fibre and occasional thin cross walls appear dividing the cell cavity.

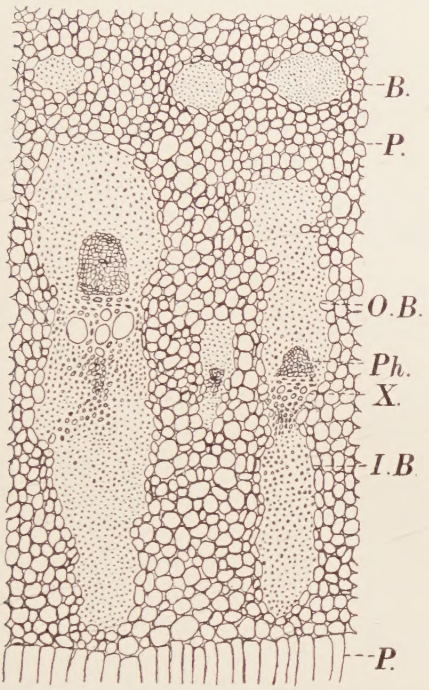


Fig. 3. Transverse section showing three fibro-vascular bundles and three fibre groups. (×80.)

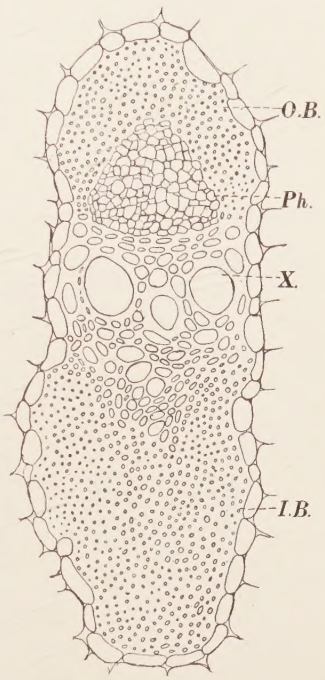


Fig. 4. Transverse section of single fibro-vascular bundle from near tip of leaf. (×140.)

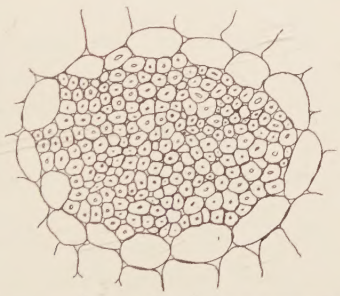


Fig. 5. Transverse section of one of the groups of fibres. (×270.)

The fibres range in length from 2·8 mm. to 6·8 mm. and in width from ·008 mm. to ·027 mm. The average relation of width to length is as 1 to 565.

The lumen varies from 1 μ to 16 μ in diameter.

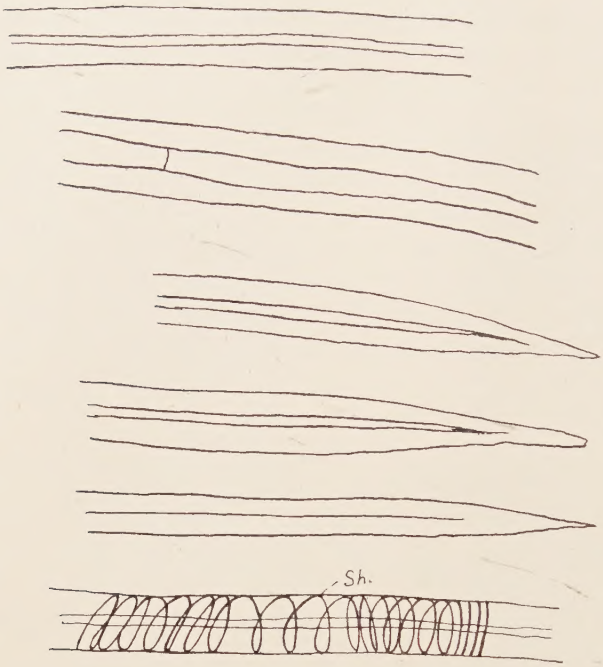


Fig. 6.

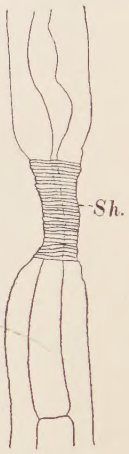


Fig. 7.

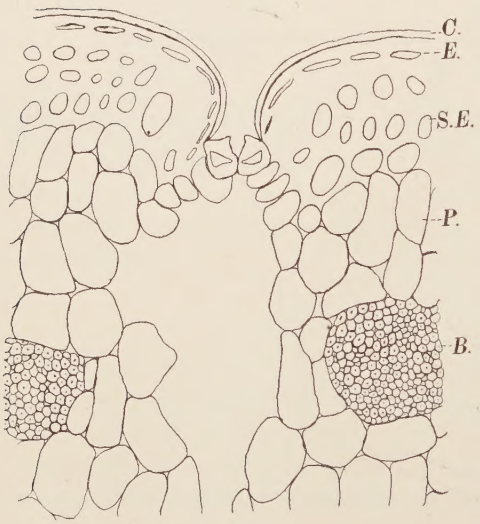


Fig. 8.

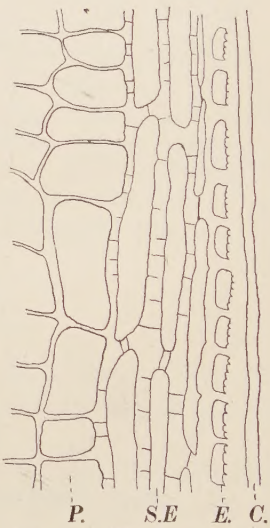


Fig. 9.

Fig. 6. Portions of fibres showing ends, lumen, and a fibre after treatment with zinc-chlor-iodine showing spiral sheath.
Fig. 7. Portion of fibre after treatment with cuprammonia.
Fig. 8. Transverse section through stoma.
Fig. 9. Longitudinal section through one of the ridges on abaxial side of leaf.

Reagents and stains.

Cuprammonia causes a swelling and final solution of the fibres, thus indicating their cellulose nature. The fibres do not swell uniformly but are constricted here and there, as if by a closely wound thread, which swells more slowly than the inner portion of the fibre. As the fibres swell, thin cross walls appear distinctly (Figs. 6 and 7). The protoplasm of the fibre remains after the solution of the cellulose wall. The crescent-shaped fibre groups around the fibro-vascular bundles are of the same structure as the fibres in groups in the parenchyma.

In using Vetillart's reagent, the sections are first stained in a solution of iodine in potassium-iodide. Then a solution of sulphuric acid made by adding the acid drop by drop to a $66\frac{2}{3}$ per cent. solution (by volume) of glycerine until the volumes of the two are equal. Sections treated with this reagent, after staining in iodine as above, show the bast fibres to be coloured blue, an indication of their cellulose composition.

Zinc-chlor-iodine. With this reagent the bast fibre and parenchyma cell walls are coloured violet. A single row of sclerenchyma surrounding the xylem and phloem is coloured yellow.

Phloroglucin. With phloroglucin and hydrochloric acid the bast fibres are coloured red, an indication of their partial lignification.

Anilin sulphate. This reagent followed by sulphuric acid colours the fibres a bright yellow, a verification of the lignified nature of the fibres.

Safranin. With this stain the bast fibre groups and fibro-vascular bundles are coloured red. The colour is easily removed from the bast fibres but is retained by the layer of mechanical cells immediately surrounding the phloem of the fibro-vascular bundles and by the xylem portion of the bundles.

Delafield's haematoxylin. This dye stains the fibres a brownish-red. As the material between the fibres stained more darkly it was thought possible that a pectic middle lamella is present, as this is specifically a stain for these substances.

Ruthenium red. As this stain is also specific for pectic substances, it was used in freshly prepared hot solution, with the result that no differences were noted between the colour of the fibres and the material surrounding them. Hence, there is probably no appreciable amount of pectin present.

Polarised light. Under polarised light the wall is not strongly doubly refractive. The fibres show light and dark areas, but the varied colours shown by flax and hemp fibres are lacking.

Dichroism. When stained with various dyes such as safranin, methylene blue or Congo red, certain fibres show dichroism when examined under a dichroic ocular. The fibres of *Neoglaziovia* were stained in this way and examined and appeared to be only feebly dichroic. A slight difference in intensity of colour could be noted, but not the different colours such as appear in the fibres of flax.

Staining reactions of leaf tissues other than fibres.

Epidermis and sub-epidermis.

As the epidermal cell walls give the same reactions with stains as those of the sub-epidermal cells, the two are considered together. The epidermal cells are thick-walled with a tangentially flattened lumen. Simple pits connect the cells with those of the sub-epidermis. The sub-epidermal cells are thick-walled and pitted, with the long diameter of the cell in the direction of the long axis of the leaf. The cells surrounding the depressed stomata are somewhat larger than the others.

The epidermal cell walls are red with Sudan III, hence are suberised. The cuticle is of a more intense red than the epidermal cell walls.

The suberised walls of the epidermis and sub-epidermis do not swell in cuprammonia, nor do they colour with phloroglucin. With safranin they are stained bright red.

Leaf sections soaked for some time in KOH (5 per cent.) and then stained with Schulze's solution, show a violet colour in the epidermal and sub-epidermal walls. This is an additional verification of the presence of suberin.

Parenchyma.

There are two principal types of parenchyma in the leaf: the rounded, isodiametric cells surrounding the bast fibre groups and vascular bundles in the abaxial part of the leaf, and the column-like cells with their long axes at right angles to the epidermis, in the adaxial part of the leaf.

The staining reactions show the parenchyma cell walls to be composed of cellulose.

Fibro-vascular bundles.

The phloem cell walls colour a deep blue at once with iodine and sulphuric acid or with zinc-chlor-iodide. They are composed of cellulose.

The cell walls of the xylem are lignified as is shown by the phloroglucin test and also by anilin sulphate.

The more or less crescent-shaped rather massive groups of fibres in the cross-section of the bundle are of the same cell-wall composition as the bast fibres in the isolated groups in the parenchyma, *i.e.* slightly lignified cellulose.

A layer of mechanical cells lying between the phloem and the group of overlying bast fibres appears to be strongly lignified. With phloroglucin and hydrochloric acid they colour a dark red. When stained by safranin and washed in acid alcohol the dye is retained by these cells after the other mechanical cells have been cleared.

Résumé.

The valuable fibre portion of this leaf consists in the fibre groups scattered through the parenchyma of the abaxial portion of the leaf and in the groups of fibres accompanying each fibro-vascular bundle.

The bast fibres are composed principally of cellulose, as indicated by their ready solubility in copper-oxide-ammonia solution, and by their violet colour when treated with zinc-chlor-iodine and blue colour with iodine and sulphuric acid.

The fibres are not pure cellulose, however, as is shown by their red colour when treated with phloroglucin, and their yellow colour when treated with anilin sulphate.

The outer part of the fibre-wall shows clear evidence of spiral structure under the action of cuprammonia. The treated fibre presents the appearance of a series of beads of unequal size. After repeated applications of the reagent, the constricting portion of the fibre swells and loosens. All portions of the fibre give the cellulose reaction with zinc-chlor-iodine, but the spiral constricting portion takes a violet colour in advance of the inner portion of the fibre and can then be seen very distinctly.

Dimensions of Fibres.

Length mm.	Width mm.	Lumen mm.
4·65	·0035	·0041
4·41	·0164	·002
6·82	·018	·0123
4·96	·0205	·0120
4·70	·0082	·0142
5·89	·0246	·0136
5·54	·0184	·015
2·8	·0102	·005
4·34	·0164	·0082
3·41	·0277	·016

ABBREVIATIONS.

E. = epidermis; *S.E.* = sub-epidermis; *B.* = bast fibre groups; *F.* = fibro-vascular bundles; *P.* = parenchyma; *O.B.* = outer bast of bundle; *I.B.* = inner bast of bundle; *Ph.* = phloem; *X.* = xylem; *Sh.* = sheath around fibres; *C.* = cuticle.

Imperial College of Science and Technology,
London, S.W. 7

(*Received March 6th*, 1925.)

SOME INFECTION EXPERIMENTS WITH LOOSE AND COVERED SMUTS OF OATS WHICH INDICATE THE EXISTENCE IN THEM OF BIOLOGICAL SPECIES

By KATHLEEN SAMPSON, M.Sc. (Lond.).
University College of Wales, Aberystwyth.

(With 2 Text-figures.)

CONTENTS.

PAGE

I. Introduction 314
II. Scheme of experiments in Wales 315
III. Results with the two biological species of Loose Smut, *U. Avenae* (Pers.) Jens. 316
IV. Results with the two biological species of Covered Smut, *U. levis* (K. and S.) Magn. 319
V. Discussion of results 320
(*a*) Loose Smut 320
(*b*) Covered Smut 321
VI. The behaviour of different lots of *Avena strigosa* in regard to infection by Covered Smut 324
VII. Summary 325
VIII. References 325

I. Introduction.

Apart from the results of the infection experiments described in the present paper, Loose Smut, *Ustilago avenae* (Pers.) Jens., has been recorded at the Welsh Plant Breeding Station, Aberystwyth, on 31 varieties of oats; Covered Smut, *U. levis* (K. and S.) Magn., on the other hand, on three only (5). In a preliminary experiment in 1922, seed of eight varieties of oats was contaminated heavily with spores of *U. levis* (collected from Orkney *strigosa*, *Avena strigosa*, sub-sp. *orcadensis*) and sown in rows on the farm. Negative results were obtained with six varieties of *A. sativa* and with Algerian *sterilis*, but good infection was obtained with Orkney *strigosa*, the variety from which the spore collection had orginally been made.

In 1920 a paper was published by G. M. Reed, in Missouri, giving the results of infection experiments with Loose and Covered Smuts of oats for the years 1915–19 (3). These results showed certain striking differences when compared with the results obtained in Wales. For example, three lots of *A. strigosa* tested in Missouri remained entirely free from infection by *U. levis*, while in Wales this host was heavily attacked by this species. In order to make a further comparison between the Missouri and the Welsh strains of the Oat Smuts, exchange of spore collections was made. At the same time, by the kindness of Dr Reed, the writer received 33 lots of oats which had already been tested in Missouri for resistance to Loose and Covered Smuts, and a number of oat varieties were sent to Missouri from the Welsh Plant Breeding Station.

The results obtained in Missouri have been published and discussed by Dr Reed in a recent paper (4). In 1923 and 1924, the Missouri and Welsh spore material gave consistently different results on certain varieties of oats in Missouri and Dr Reed concludes that in the case of each of the two smuts, *U. levis* and *U. avenae*, two quite distinct biological species (or "physiologic races" as he calls them) exist. This is the first precise record of such specialisation among the smuts of oats. That biological specialisation does exist in the Ustilaginaceae has previously been proved experimentally by Zillig for *U. violacea* (7) and by Faris for *U. hordei* (1,2).

The four biological species of smuts were also compared during 1923 and 1924 on 45 oat varieties at Aberystwyth. The results are given below, and compared with those obtained in Missouri by Dr Reed (4). In neither season were the conditions in Wales favourable to smut attack in oats, as was shown by the comparatively small amount of smut present in farm crops in the district. The trials gave a high proportion of negative results, but the positive results which were obtained, particularly in the case of the two biological species of Covered Smut (*U. levis*), show a very close agreement with those obtained in Missouri, and they confirm the conclusion that biological species of *U. levis*, at any rate, do exist.

II. Scheme of Experiments in Wales.

45 lots of oats, comprising 38 different varieties, were tested, the following species being included: *Avena brevis* (2 lots), *A. strigosa* (5 lots), *A. sativa* (33 lots), *A. sterilis* (5 lots).

The Welsh lots of grain, having been collected with no precautions in regard to natural smut contamination, were treated as follows: Each lot was first soaked for ten minutes in formalin (1 part in 320 parts of

water), drained, left in damp filter paper for four hours and then spread out to dry. The following day each lot was washed for 10–15 minutes in clean tap-water and dried thoroughly before being stored in new paper envelopes. The Missouri lots of grain were not so treated; but control rows of these as well as of the Welsh lots, sown without previous artificial contamination with smut spores, remained free from infection in both seasons' experiments.

For sowing, four portions, of 120 grains from each lot, were placed separately in round bottom flasks and shaken thoroughly with a certain quantity (about 0·2 g.) of smut spores of the four biological species of *Ustilago* designated A, B, C, D. The origin of these spores was as follows:

A. *U. avenae*, collected Aberystwyth, 1923, ex *A. sativa* var. Potato; 1924, ex *A. sativa*, Swedish Black and a few other varieties.

B. *U. avenae*, ex Missouri.

C. *U. levis*, collected Aberystwyth, 1923 and 1924, ex *A. strigosa* sub-sp. *orcadensis*.

D. *U. levis*, ex Missouri.

After thorough dusting, the grain, together with surplus spores which had not adhered to it, was dropped in each case into a clean paper envelope from which it was subsequently sown. In order to avoid chance contamination, only one biological species of smut was dealt with on any particular day, and the dusting operations were carried out in different rooms of the building.

All the lots were sown on the same day, 120 grains per 5 foot row. The dates and places of sowing were as follows:

1923. 5th April. Penglais Field.
1924. 17th March. Gorse Field.

The "take" was in nearly all cases good. The straw was tall and "lodged" somewhat badly in both seasons.

III. Results with the two biological species of Loose Smut, *U. avenae* (Pers.) Jens.

In no single variety, either in 1923 or 1924, was a heavy infection obtained with either biological species of Loose Smut. The highest figure (approx. 12 per cent.) was given by Ceirch du Bach affected with the Welsh biological species in 1924. The results may be summarised as follows:

(1) *Varieties showing slight infection with both biological species, i.e. Welsh and Missouri.*

A. sativa	Potato	(1029)*	1923 and 1924
,,	Early Champion	(2388)	1924
,,	Victor	(2394)	,,

* See footnote, p. 319.

(2) *Varieties showing slight infection with Wales Loose Smut, none with that from Missouri.*

A. sativa	Record	(1026)	1924
,,	Rad. Sprig	(1078)	,,
,,	Ceirch du Bach	(1080)	,,
,,	Golden Drop	(2389)	,,
,,	June	(2390)	,,
,,	Black Diamond	(2396)	,,
,,	Tobolsk	(2403)	,,
,,	Canadian	(2387)	1923

(3) *Varieties showing slight infection with Missouri Loose Smut, none with that from Wales.*

A. sativa	Awnless Probsteier	(2385)	1924
,,	Danish	(2397)	,,
,,	Green Russian	(2400)	,,
A. strigosa sub-sp. *orcadensis*		(521)	1923
,, ,, *glabrescens*		(363)	,,
,, ,, *pilosa*		(362)	,,

(4) *Varieties showing no infection with either biological species.*

A. sativa var.	Black Mesdag	(722)	1923 and 1924
,,	,, ,,	(1079)	,, ,,
,,	,, ,,	(1610)	,, ,,
,,	,, ,,	(2371)	1924
,,	Caucasus	(2372)	,,
,,	Danish Island	(2373)	,,
,,	Black Diamond	(2374)	,,
,,	Monarch	(2375) and (2402)	,,
,,	Pringle Progress	(2376)	,,
,,	Siberian	(2377)	,,
,,	Belyak	(2386)	,,
,,	Kherson Selection	(2391)	,,
,,	North Finnish	(2392)	,,
,,	60 Day	(2393)	,,
,,	Aurora	(2395)	,,
,,	Japan	(2398)	,,
,,	Scottish Chief	(2399)	,,
,,	Ligowo	(2401)	,,
A. sterilis var.	Burt	(2378)	,,
,,	Fulghum	(2379) and (2380)	,,
,,	Red Rustproof	(2381)	,,
A. brevis		(2383) and (2384)	,,
A. strigosa sub-sp. *glabrescens*		(2382) and (1096)	,, 1923 and 1924

Cc 2394. *Avena sativa* var. Victor. *D*, panicle affected by *U. levis* ex Missouri. *A* and *B*, panicles affected by *U. avenae* ex Missouri. *A*, recently exserted. *B*, exserted several days previous to cutting; loose spores have blown away.

Cc 2389. *Avena sativa* var. Golden Drop. *A*, *Ustilago avenae* ex Aberystwyth. *D*, *U. levis* ex Missouri.

Ustilago avenae and *U. levis* on the same variety of oat.

IV. Results with the two biological species of Covered Smut, *U. levis* (K. and S.) Magn.

Good infection was obtained with some varieties in both seasons. Those lots which produced affected panicles in one or both seasons are listed in Table I. The following varieties were tested with negative results:

1923 and 1924. *A. sativa*, var. Black Mesdag (722 and 1079)*.

1924. *A. sativa*, vars. Black Mesdag (2371), Caucasus (2372), Danish Island (2373), Black Diamond (2374) and (2396), Monarch (2375), Pringle Progress (2376), Siberian (2377), Early Champion (2388), June (2390), Kherson Selection (2391), 60 Day (2393), Aurora (2395), Danish (2397), Japan (2398), Scottish Chief (2399), Green Russian (2400), Ligowo (2401) and Tobolsk (2403).

A. sterilis, vars. Burt (2378), Fulghum (2379 and 2380), Red Rustproof (2381).

A. strigosa sub-sp. *glabrescens* (2382).

* The numbers in brackets after each variety indicate the station numbers.

Table I.

Comparison of Infection by two Biological Species of U. levis (*K. and S.*) *Magn.*

Species and variety	Sta. no.	Wales material 1923 Total no. Tillers	1923 No. inf.	1923 % inf.	Wales material 1924 Total no. Tillers	1924 No. inf.	1924 % inf.	Missouri material 1923 Total no. Tillers	1923 No. inf.	1923 % inf.	Missouri material 1924 Total no. Tillers	1924 No. inf.	1924 % inf.
A. brevis Roth.	2383	36	12	33	—	0	0	not tested			—	0	0
,, ,,	2384	22	8	36	200	134	67	,,		,,	—	0	0
A. strigosa Schreb. sub-sp. *glabrescens*	2382	95	0	0	—	0	0	,,		,,	—	0	0
,, ,,	1096	247	9	4	115	19	17	—	0	0	162	4	2
,, ,,	363	183	142	53	96	50	52	218	87	40	—	0	0
,, *pilosa*	362	257	150	58	127	48	38	239	19	8	—	0	0
,, *orcadensis*	521	167	105	63	63	28	44	237	66	28	53	3	6
A. sativa L. var. Potato	1092	—	0	—	—	1	—	129	21	16	—	0	—
,, Ceirch du Bach	1080	—	0	—	—	0	—	147	17	12	—	2	—
,, Radnorshire Sprig	1078	—	0	—	—	0	—	148	15	10	—	1	—
,, Victor	2394	—	0	—	—	0	—	not tested			—	7	—
,, Golden Drop	2389	—	0	—	—	0	—	,,		,,	—	2	—
,, North Finnish	2392	—	0	—	—	0	—	,,		,,	—	2	—
,, Monarch	2402	—	0	—	—	0	—	,,		,,	—	1	—
,, Record	1026	—	0	—	—	0	—	—	4	—	—	0	—
,, Black Mesdag	1610	—	1	—	—	0	—	—	0	—	—	0	—
,, Awnless Probsteier	2385	—	0	—	—	0	—	not tested			—	1	—
,, Belyak	2386	—	0	—	—	0	—	,,		,,	—	3	—
,, Canadian	2387	—	0	—	—	0	—	,,		,,	—	1	—
A. sterilis L. var. Red Algerian	1613	—	2	—	—	0	—	—	0	—	—	0	—

The percentage infection was estimated by tiller counts in 1923, and is therefore given on this basis also for 1924. When the grain is sown in rows and a good stand occurs, the relative percentages obtained by tiller enumeration agree closely with those based on a count of smutted plants, as is shown by the following results obtained in 1924:

% Smut based on:							
(1) Number of smutted tillers	38	52	44	6	17	2	67
(2) Number of smutted plants	38	56	49	8	18	4	66

Owing to the difficulties of harvesting in the wet seasons 1923 and 1924, only such rows were actually cut, harvested and counted as showed an appreciable percentage smut. In other cases, the smutted panicles were collected and recorded as and when they appeared in the field. For this reason, it has not been possible to include in the above table the percentage smut for all varieties actually showing infection. It is unlikely that it would in such cases be more than 5 per cent.

V. Discussion of results.

(*a*) *Loose Smut.*

The amount of infection in that part of the experiment dealing with the two biological species of Loose Smut was too small in both seasons to admit of reliable conclusions in regard to their behaviour on different oat varieties, but the results present a few points which are of interest in comparison with those obtained in Missouri (4). Dr Reed states that "the most significant differences between the Loose Smut from Missouri and that from Wales are found in the failure of the latter to cause severe infection of *A. nuda* (S.N. 30) and of the Welsh varieties of *A. strigosa* (S.N. 587, 589 and 592)." Approximately, 100 per cent. infection was obtained by Dr Reed on these varieties with the biological species from Missouri. In Wales, the three sub-species of *A. strigosa*, *orcadensis*, *glabrescens* and *pilosa* were the only varieties except Potato infected by the Missouri biological species of Loose Smut in 1923, but they gave negative results with the biological species from Wales in both seasons.

Further agreement with the Missouri results is afforded by the variety Potato. In Missouri in 1924, this variety gave 100 per cent. infection with both biological species of Loose Smut. In Wales, this was the only variety which gave a positive result with the two biological species in both seasons. It is interesting to find that this variety is susceptible to the Missouri biological species of smut, since it has repeatedly been observed at the Welsh Plant Breeding Station oat trials as one of the varieties most

severely attacked by Loose Smut. Another variety which in 1924 gave in Missouri 100 per cent. infection with the two biological species of smut is Irish Tan. This variety was not included in the Welsh experiments under discussion here, but, like Potato, it has in former years shown very high percentages of Loose Smut in the oat trials.

In a previous paper (5), where reference was made to the occurrence of Loose Smut on a large number of different oat varieties, Black Mesdag, a variety of *A. sativa*, was listed among fifteen varieties which showed over 4000 smutted panicles per acre. Since it was realised that the plots from which the smut collections were made might perhaps not be pure for a particular variety, the data were given only as an indication of the wide range of Loose Smut among oat varieties and the inclusion of a variety in this list was not considered as an absolute guarantee of its susceptibility to the disease[1]. Black Mesdag is perhaps a case in point and the apparent susceptibility of this variety in 1921 was possibly due to rogues, since two lots of this variety tested with the Welsh biological species of Loose Smut in Missouri gave negative results in 1923 and 1924, and four lots of the same variety tested in Wales also gave negative results in the two seasons. The same variety has also given negative results in tests made at both centres with the Missouri biological species of smut. It is a variety meriting further study from this point of view, since few varieties of *A. sativa* have shown resistance to this disease consistently when tested for several years or at different centres.

(*b*) *Covered Smut.*

As already stated, twenty-three varieties of oats gave negative results with Covered Smut, but in both seasons certain varieties showed good infection. In 1923, *A. strigosa* sub-sp. *orcadensis* showed the highest figure (63 per cent.) and in 1924, 67 per cent. infection was shown by one lot of *A. brevis*. Both these results were obtained with the Welsh biological species of smut.

Varieties of *Avena* behave very differently towards the biological species of Loose and Covered Smuts so far studied in Wales. Reed, after five seasons' study at Brooklyn, concluded in 1919 that "no apparent differences in the infection capacity of the two species of smuts may be noted" (3). More recent experiments conducted in Missouri (4) certainly prove that the Missouri biological species of Loose and Covered Smut do not show the marked differences which are shown by the two Welsh species. Speaking generally, the results both in Wales and in Missouri

[1] See (5), page 56, footnote.

indicate that the Welsh biological species of *U. levis* is capable of causing very severe infection in varieties of the species *brevis* and *strigosa*, but that many varieties of *sativa* are resistant to it. On the other hand, the Welsh biological species of *U. avenae* produces high percentages of infection in varieties of *A. sativa* but has given negative results with several lots of *A. brevis* and *A. strigosa*.

That the Welsh and Missouri biological species of *U. levis* are distinct is brought out both in Missouri and in Wales by their behaviour on *A. brevis* and certain lots of *A. strigosa*, the results at the two stations showing good agreement. These results are set out in Table II where a number of tests with different lots of *A. brevis* and *A. strigosa* are collected together.

Previous to 1924, *A. brevis* gave negative results with *U. levis* in Missouri, but both in Missouri and in Wales a high percentage of infection was obtained on *A. brevis* with the Welsh species. On certain lots of the three sub-sp. of *A. strigosa*, the Welsh biological species of *U. levis* gave a higher percentage infection than the Missouri species, and this again was noticed at both places of experiment.

The results with *sativa* varieties also show a certain amount of agreement in Missouri and in Wales. Reed obtained negative results with several *sativa* vars. contaminated with the Welsh biological species of *U. levis* and good infection (100 per cent. in case of Potato) with the Missouri species. At Aberystwyth, several *sativa* varieties gave negative results in 1922, 1923 and 1924 with Welsh Covered Smut, but in 1923 Potato, Ceirch du Bach, and Radnorshire Sprig gave 16, 12 and 10 per cent. infection respectively with the Missouri species.

That the two biological species of *U. levis* under experiment show quite distinct powers of causing infection in certain varieties of oat is therefore confirmed by the Aberystwyth trials. It should be noticed that the results here discussed are in harmony with generally accepted facts in regard to the distribution of these two smuts of oats in the British Isles and the U.S.A. In the British Isles, the more widely distributed smut is *U. avenae* which can attack a large number of varieties of *A. sativa*, the species usually grown in this country. *U. levis*, however, which appears to have only a very limited power of attacking *sativa* var. is apparently rare in Britain[1]. In the U.S.A. it appears that *U. levis* causes at least as much damage to the oat crop as *U. avenae* (6). A strain of *U. levis* exists there which has the power of causing heavy infection in a number of

[1] As far as the writer is aware no systematic search for Covered Smut of oats has been made in the British Isles.

Table II.

Percentage Infection of different lots of A. strigosa *and* A. brevis *by two collections of* U. levis. *Results in Missouri and at Aberystwyth.*

Variety	Missouri Station No. S.N.	Aberystwyth Station No. Cc	*U. levis* spores from Missouri: Tests at Missouri 1915	1916	1918	1919	1924	*U. levis* spores from Missouri: Tests at Aberystwyth 1923	1924	*U. levis* spores from Wales: Missouri 1923	1924	*U. levis* spores from Wales: Aberystwyth 1922	1923	1924
A. brevis (wild type)	289	2384	—	—	—	—	2·5	—	0	—	100	—	36	67
,,	77	2383	—	0	0	0	—	—	0	—	—	—	33	0
,,	1	—	0	0	—	—	—	—	—	—	—	—	—	—
,,	134	—	—	—	0	0	—	—	—	—	—	—	—	—
A. strigosa, sub-sp. *glabrescens*	133	—	—	0	0	—	0	—	—	—	0	—	—	—
,,	29	2382	0	0	0	0	—	—	0	—	—	—	0	0
,,	586	—	—	—	—	—	—	—	—	0	—	—	—	—
var. *cambrica*	—	1096*	—	—	—	—	—	0	2	—	—	0	4	17
,, *cambrica*	588	1709†	—	—	—	—	—	—	—	0	—	—	—	—
,, *albida*	587	1652†	—	—	—	—	46	—	—	83	100	—	—	—
,, *cambrica*	—	363†	—	—	—	—	—	40	0	—	—	—	53	56
A. strigosa, sub-sp. *pilosa* var. *fusca*	592	362†	—	—	—	—	87	8	3	100	100	—	58	38
,, *fusca*	593	1651†	—	—	—	—	—	—	—	80	—	—	—	—
A. strigosa, sub-sp. *orcadensis*	—	521†	—	—	—	—	—	28	6	—	—	50	63	49
var. *flava*	589	—	—	—	—	—	100	—	—	—	100	—	—	—
,, *nigra*	591	1687†	—	—	—	—	65	—	—	83	—	—	—	—

* Known to contain a few plants of *A. strigosa*, sub-sp. *orcadensis*.

† Pure line.

varieties of *A. sativa*. Thus it appears that the relative importance of the two smuts of oats in the two countries may perhaps be related to the geographical distribution of the two different biological species; but, as Reed suggests, it is quite likely that closer study will reveal other biological species in the same locality or perhaps even in the same field.

VI. The behaviour of different lots of *A. strigosa* in regard to infection by Covered Smut

In Table II the results with both strains of Covered Smut on a number of lots of *A. strigosa* and *A. brevis* have been tabulated. It is evident that wide differences in regard to susceptibility do exist among different lots of *A. strigosa*, and perhaps also of *A. brevis*. Thus in the case of *A. strigosa*, sub-sp. *glabrescens*, one lot (S.N. 29, Cc 2382) gave negative results four years in succession when tested in Missouri with the native biological species, further, no infection at Aberystwyth in 1924 with the Missouri strain, and finally a similar result in 1923 and 1924 when tested at Aberystwyth with the Welsh biological species. Negative results were also obtained with the Welsh biological species on S.N. 133, 586 and 588, whilst Cc 1096 has consistently shown resistance. These again are lots of the sub-species *glabrescens*. Two lots, on the other hand (Cc 1652 and Cc 363), appear to be susceptible to both biological species of Covered Smut. Investigations in progress at the Welsh Plant Breeding Station have already shown that a considerable amount of variation exists in the sub-species *glabrescens*, not only in physiological but also in well-marked morphological characters[1]. It is not yet known if any of these characters are correlated with resistance or susceptibility to smut, but the above data serve to emphasise the importance of using pure lines in disease resistance studies and of testing as far as possible a number of lines of each variety. It is hoped in the coming season to expand the trials with the oat smuts to include a large number of the pure lines of *A. strigosa* which are under study at the Welsh Plant Breeding Station.

In conclusion, the writer desires to thank Professor Stapledon for so kindly placing at her disposal the facilities of the Welsh Plant Breeding Station and for his interest in the work.

Thanks are due also to Mr D. W. Davies, Advisory Mycologist, for valuable help in connection with the photographs and harvesting operations.

[1] Unpublished data, kindly supplied by Mr E. T. Jones, who is in charge of Breeding Experiments with Oats.

Grateful acknowledgement is made to Dr G. H. Pethybridge for reading the manuscript and for his helpful criticism of the paper.

VII. SUMMARY.

1. Infection experiments are described with two spore collections of *U. avenae* (Pers.) Jens. and *U. levis* (K. and S.) Magn. obtained from Missouri and from Wales.

2. The results with *U. levis* indicate that the spore collections under test represent two distinct biological species, thus confirming the conclusion arrived at by Dr Reed(4), working with the same material at Missouri.

3. Forms highly susceptible and resistant to *U. levis* appear to exist in the sub-species *glabrescens* of *A. strigosa*.

VIII. REFERENCES.

(1) FARIS, J. A. (1924). Factors influencing Infection of *Hordeum sativum* by *Ustilago hordei*. *Amer. Journ. Bot.* XI, 189–214.

(2) —— (1924). Physiological Specialization of *Ustilago hordei*. *Phytopathology*, XIV, no. 12, 537–557.

(3) REED, G. M. (1920). Varietal Resistance and Susceptibility of Oats to Powdery Mildew, Crown Rust and Smuts. *Missouri Agric. Sta. Res. Bull.* XXXVII, 1–41.

(4) —— (1924). Physiologic Races of Oat Smuts. *American Journal of Botany*, XI, 483–492.

(5) SAMPSON, KATHLEEN and DAVIES, D. W. (1923). Incidence of Fungus Diseases on Oat Varieties in the Seasons 1921–22. *Welsh Plant Breeding Station Bulletin*, Series C, no. 3, p. 56.

(6) STANTON, T. R., STEPHENS, D. E. and GAINES, E. F. (1924). Markton, an Oat variety immune from Covered Smut. *U.S.A. Dept. Agric. Washington Dept. Circular* 324.

(7) ZILLIG, H. (1921). Über Spezialisierte Formen beim Antherbrand (*Ustilago violacea* (Pers.) Tuck). *Centralbl. Bakt.* II, no. 53, 33–73.

(*Received February 13th*, 1925.)

NOTE ON THE BACTERIAL SOFT ROT OF TURNIPS

By H. WORMALD and R. V. HARRIS.
(*Horticultural Research Station, East Malling, Kent.*)

A bacterial soft rot of turnips was recorded for this country by Potter (10,11) in 1899, the organism causing it being described as a *Pseudomonas* with a single polar flagellum and named, as a new species, *Ps. destructans*. Some years later Harding and Morse (2) obtained from Potter, not his original strain, which had not been kept, but a freshly isolated culture which proved to be an organism with peritrichous flagella. In 1909 they received another freshly isolated culture from the same source and this also was found to be a *Bacillus*. It would appear then that, whatever Potter's orginal organism was, he had on two subsequent occasions isolated from turnips an organism with peritrichous flagella. Harding and Morse found that "Potter's Bacillus" showed no essential difference from the type strain of *Bacillus carotovorus* isolated from carrots by L. R. Jones (6,7).

A bacterial rot of turnips was reported by Carruthers and Smith (1) in 1901, but a detailed description of the organism was not given and there was no reference to the number of its flagella.

In 1910 Johnson and Adams (4) ascribed a soft white rot of turnips in Ireland to the action of Potter's *Pseudomonas destructans*, stating that "the bacterium has a single polar flagellum." They found, however, that it had many characters in common with *Bacillus oleraceae*, an organism isolated by Harrison (3) from cauliflower, turnip and cabbage in Canada. Harding and Morse include *Bacillus oleraceae* under *Bacillus carotovorus* Jones.

Priestley and Lechmere (12), describing a rot of swedes in 1910, found that the organism they isolated also agreed closely with *Pseudomonas destructans* and *Bacillus oleraceae*, but in this case peritrichous flagella were found.

Paine (8) examined a culture named *Pseudomonas destructans* obtained from Kràl's collection and found on a first trial uniflagellate rods only, but later was able to demonstrate the presence of rods, some uniflagellate, others with peritrichous flagella. He suggested, therefore, that the name *Pseudomonas destructans* should be abolished "until a definitely uni-

flagellate organism answering exactly to the description given by Potter shall be again obtained from rotted turnips or other tissue."

More recently, however, a rot of turnips in Wales has been studied by S. G. Jones (5) who concludes that it is caused by a varietal form of *Pseudomonas destructans*: the organism had a single long polar flagellum and "no variation in the number or situation of the flagella was seen[1]."

It would appear, then, that in the British Isles, soft rot bacteria staining as *Pseudomonas* have been isolated from turnips on three occasions (Potter, Johnson and Adams, S. G. Jones) and others staining as *Bacillus* also on three occasions (Potter (twice), Priestley and Lechmere), the number of flagella in the case of Carruthers and Smith's organism not being ascertained.

In the autumn of 1923 a rot destroying nearly all the turnips on a small plot in a private garden at East Malling came to the notice of the writers. The disease started at the "crown," suggesting that it was the young leaves that became primarily infected. The rot extended downward from the crown until the whole root was involved and finally the rotted parenchymatous tissues disappeared, leaving the vascular cylinder only as a fibrous network. Sometimes almost complete specimens of the "skeletonised" vascular system were found.

The disease may be described as a "white rot" as there was no definite browning of the internal rotting mass; the marginal region of the advancing infected portion on partially rotted turnips, as seen on the exterior of the root, was, however, a light greyish brown.

From a partly rotted root an organism was isolated which induced a rotting of fresh turnip slices; it was re-isolated on several occasions and later was shown to infect young turnip leaves readily, the rot extending into the crown and later destroying the whole root. This organism was stained to show flagella according to the method recommended by Plimmer and Paine (9) and no difficulty was experienced in demonstrating the presence of a number of peritrichous flagella (6 to 11). The usual cultural tests were carried out to determine its physiological reactions and the results, summarised, are as follows: The colour of agar streaks and colonies is white but somewhat yellowish on steamed potato[2]; gelatine is liquefied readily; glucose, saccharose and lactose with peptone water

[1] A rot of radishes in Japan is reported (13) to be caused by an organism corresponding in form and behaviour in culture media to *Pseudomonas destructans* Potter.

[2] On white solid media such as steamed potato the yellowish colour produced by *Bacillus carotovorus* is unmistakeable, but for organisms of this type the colour is usually referred to as "white." (See E. F. Smith's *An Introduction to Bacterial Diseases of Plants*, footnote, p 230.)

in fermentation tubes yield acid with the evolution of gas in each case; potassium nitrate in broth is reduced to nitrite; peptone water containing glycerine becomes acidified without visible evidence of evolution of gas; there is slight diastasic action on potato starch; growth in Uschinsky's solution is vigorous with the formation of a pellicle.

These results are similar to those obtained for *Bacillus carotovorus* by Jones (6), and for the soft rot organism of celery, also referred to the same species, as described by one of the present writers (14,15), and they call for no special notice except perhaps with respect to the behaviour in the sugar solutions in fermentation tubes. Harding and Morse, who gave special attention to the reaction of the soft rot organisms in fermentation tubes containing dextrose, lactose, and saccharose respectively, found that the type strain of *Bacillus carotovorus* almost invariably produced gas from each of these three sugars (group number for this series of tests ·111). "Potter's Bacillus," in their hands, produced gas from lactose but not from dextrose or saccharose (·212). Another turnip bacillus which they studied, "Turnip Rot D," did not produce gas in any of these sugars (·222). The organism *Bacillus oleraceae*, isolated by Harrison from cauliflower, turnip, and cabbage, was also tested by Harding and Morse, but the host of the particular strain they used is not stated; the results they obtained from this strain in fermentation tubes were variable for dextrose and saccharose, gas being liberated in some tests but not in others, while from lactose gas appeared in all the tests (·112; ·111; or ·211). The turnip bacillus which Priestley and Lechmere examined "gives off gas during fermentation," but no further details were given. Turnip-rot bacilli have therefore given variable results in the fermentation tests, and the results obtained by the writers were likewise not wholly consistent.

The strain examined at East Malling was tested for the fermentation reactions soon after isolation and was found to produce gas from lactose but not from dextrose or saccharose, and on repeating the test the same result was obtained (·212); it behaved then as "Potter's Bacillus." On re-isolating, however, after inoculating the same strain into living turnip stems and fresh sterile slices of turnip (three separate isolations being made and tested) gas was obtained from each of the three sugars in every test (·111), results similar to those obtained by Harding and Morse for *B. carotovorus* Jones and by one of the writers (15) for a celery-rot strain of the same species.

The turnip-rot organism isolated at East Malling, in regard to its fermentation reaction with the sugars tested, and in the other tests carried out, is therefore indistinguishable from *Bacillus carotovorus* Jones, and has the same group number, viz. 221·1113022.

Summary.

A soft rot of turnips in a garden at East Malling was found to be caused by an organism which has peritrichous flagella and has the same group number as *Bacillus carotovorus* Jones.

REFERENCES.

(1) Carruthers, W. and Smith, A. L. (1901). A Disease of Turnips caused by Bacteria. *Journ. Bot.* xxxix, 33.

(2) Harding, H. A. and Morse, W. J. (Nov. 1909). The Bacterial Soft Rots of Certain Vegetables. I. The Mutual Relationships of the Causal Organisms. *New York Agric. Expt. Sta. Bull.* 11, 251–287.

(3) Harrison, F. C. (1904). A bacterial disease of Cauliflower (*Brassica oleracea*) and allied plants. *Cent. f. Bakt.* 2 Abt. xiii, 46–55, 185–198.

(4) Johnson, T. and Adams, J. (Feb. 1910). Bacterial Rot in Turnips and other Brassicas in Ireland. *Econ. Proc. Roy. Dublin Soc.* ii, no. 1, 1–7.

(5) Jones, S. G. (Apr. 1922). A Bacterial Disease of Turnips (*Brassica napus*). *Journ. Agric. Sci.* xii, pt. 2, 292–305.

(6) Jones, L. R. (1900). A Soft Rot of Carrot and other Vegetables. *Vermont Agric. Expt. Sta. 13th Ann. Rep.* 299–332.

(7) —— (1901). *Bacillus carotovorus* n. sp. die Ursache einer weichen Fäulnis der Möhre. *Cent. f. Bakt.* 2 Abt. vii, 12–21, 61–68.

(8) Paine, S. G. (July, 1918). An Epitome of Bacterial Diseases of Plants in Great Britain and Ireland. *Ann. Appl. Biol.* v, no. 1, 62–76.

(9) Plimmer, H. G. and Paine, S. G. (1921). A New Method for the Staining of Bacterial Flagella. *Journ. Path. and Bact.* xxiv, 286–288.

(10) Potter, M. C. (Nov. 1899). On a Bacterial Disease—White Rot—of the Turnip. *Proc. Univ. of Durham Phil. Soc.*

(11) —— (1901). On a Bacterial Disease of the Turnip (*Brassica napus*). *Proc. Roy. Soc.* lxvii, 442–459.

(12) Priestley, J. H. and Lechmere, A. E. (Dec. 1910). A Bacterial Disease of Swedes. *Journ. Agric. Sci.* iii, pt. 4, 390–397.

(13) Takimoto, S. (1921). Studies on the putrefaction of vegetables (Japanese). *Journ. Plant Protect.* viii, 344–353. [See *Bot. Abstracts*, xiv, no. 1 (Jan. 1925), 91.]

(14) Wormald, H. (May, 1914). A Bacterial Rot of Celery. *Journ. Agric. Sci.* vi, pt. 2, 203–219.

(15) —— (Mar. 1917). The Celery Rot Bacillus. *Journ. Agric. Sci.* viii, pt. 2, 216–245.

(*Received May 9th*, 1925.)

A PHYSIOLOGICAL STUDY OF THE COMMON BACTERIA OF AIR, SOIL AND WATER WITH A VIEW TO THEIR EXACT DIAGNOSIS

By MANECK M. MEHTA,
Wilson College, Bombay, India.
(*From the Department of Plant Physiology and Pathology, Imperial College of Science, London.*)

CONTENTS.

	PAGE		PAGE
Methods	330	*Sarcina lutea*	347
Microscopic examination	334	*S. flava*	348
Thermal death point	337	*Sarcina* (species from milk)	348
Bibliography	339	*S. aurantiaca*	348
Bacillus coli communis	339	*Bacillus anthracoides*	348
B. coli communior	340	*B. mycoides*	349
B. coli mutabilis	340	*B. subtilis*	350
B. prodigiosus	341	*B. megatherium*	351
B. violaceus	342	*B. mesentericus*	352
Pseudomonas fluorescens liquefaciens	342	*B. carotovorus*	353
Ps. fluorescens non-liquefaciens	343	*B. solanisaprus*	353
Ps. pyocyaneus	344	*B. phytophthorus*	354
Bacillus proteus vulgaris	345	*Pseudomonas campestris*	355
B. proteus mirabilis	345	*Ps. lathyri*	358
B. proteus Zopfius Zenkeri	346	Tables	356

METHODS.

In the following pages an endeavour has been made to arrange concisely the methods used for the study of the common bacteria of air, soil, and water, together with the experimental results. Details of technique have been given in order to facilitate the work of repetition by others.

Media.

The way in which colonies develop upon a standard medium, the form which they assume, and the chemical action which they have upon the medium, are of the greatest importance in distinguishing different species. The same species may exhibit many points of difference when

grown upon two media which are very nearly alike in composition and acidity. The want of uniformity in the ingredients (peptone and beef extract), frequently used for the preparation of nutrient media gives rise to great difficulty in comparing the work and conclusions of those who have carried on investigations in the subject. To avoid this difficulty it was considered advisable to prepare a standard synthetic medium.

Series of experiments were carried out by adding different proportions of asparagin, sodium nitrate, magnesium sulphate, calcium chloride, potassium hydrogen phosphate and ammonium lactate to 1 per cent. starch solution. All these media when heated turned strongly acid and were unsatisfactory in view of the fact that after neutralisation a precipitate was produced on sterilisation.

Ammonium lactate was therefore excluded in one series and alkaline potassium phosphate substituted for potassium hydrogen phosphate in another series, but none of these media gave satisfactory results. The starch present constantly caused turbidity even after filtration, and, further, the media were unsatisfactory in that they did not allow of any uniformity in the amount of growth of the different species under investigation.

In place of the starch, potato extract was substituted and all the above sets of experiments were repeated, but with practically the same unsatisfactory results.

Most of the ingredients necessary for bacterial growth, viz. protein material and inorganic salts, are present in lentil seeds. A fresh series of experiments was started, using lentil as the chief ingredient.

Balland(1) and Church(2) give the following analysis of the seeds of *Lens esculenta*:

		Church	
	Balland	Husked	Unhusked
Water	11·7 to 13·5	11·8	11·7
Protein	20·3 „ 24·2	25·1	24·9
Fat	0·6 „ 1·5	1·3	1·5
Nitrogen-free extract	56·1 „ 62·4	58·4	56·0
Crude fibre	3·0 „ 3·6	1·2	3·6
Ash	2·0 „ 2·7	2·2	2·3

König(3) quotes as an analysis of ash:

K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	Cl
34·8	13·5	6·3	2·5	2·0	36·3	4·6

Percentage of ash in dry substance 2·07.

Nutrient Lentil Broth.

To 1100 c.c. of distilled water 60 gm. of whole lentils and 10 gm. of sodium chloride were added, mixed in a flask and steamed at 100° C. for one hour.

The liquid was filtered through "winceyette" cloth, the filtrate was brought up to 1000 c.c., it was rendered slightly but distinctly acid with 20 per cent. acetic acid, using Rosolsäure-papier as an indicator and clarified with the white of an egg by steaming for half an hour, and filtered when cold. (The addition of 2 c.c. of yolk to the egg-white brings about the coagulation fairly quickly and much facilitates the filtration of the medium.)

Hydrogen ion concentration was next determined, bromo-thymol-blue being used as an indicator, and the medium brought to 7·0 *p*H by means of sodium hydroxide solution. It was heated again for 15 minutes, filtered if necessary, tubed, and sterilised by steaming for 30 minutes on three successive days. Nutrient nitrate and sugar media were prepared from this plain lentil broth in the usual way, using 0·5 per cent. of potassium nitrate or 1·5 per cent. of glucose, lactose, or saccharose.

Since bacteria are so very susceptible to their environment, before passing to the tests of their fermentative activities, it was considered advisable to grow them for a certain length of time upon some medium which might be calculated to give the organisms no bias in the direction of the fermentation of any of the test substances. For this purpose a small quantity of each of the test sugars, of nitrate and of glycerine, were added to the lentil broth, the idea being that any organism which normally possesses the power of exerting fermentative activity in certain directions would find in the medium a favourable pabulum and thus would receive no stimulus towards a "training" in other directions.

The medium employed, therefore, consisted of: 1000 c.c. lentil broth, as described above, to which the following additions were made:

5 gm. glucose	5 gm. potassium nitrate
5 „ lactose	5 „ glycerine
5 „ saccharose	

Nutrient agar and gelatine were prepared from this lentil broth in the usual way, using 12 per cent. gelatine or 2 per cent. agar.

Hydrogen ion concentration.

Bacteria are highly susceptible to variations in the acidity and alkalinity of media. It is necessary, therefore, to adjust the reaction both

for the sake of favouring growth and in order to ensure uniformity of growth characters. Hydrogen ion concentration determination was made by colorimetric method according to Gillespie (4), using bromo-thymol-blue as indicator.

It was found that the hydrogen ion concentration of a medium was almost always shifted towards the acid side during sterilisation, especially when the medium was neutral or alkaline.

Uschinsky Solution (5).

Uschinsky solution was always distinctly acid in reaction. If sodium hydroxide solution was added till the liquid containing litmus was neutral wine-colour on heating some precipitate came down and the solution again turned acid red. The medium was brought up exactly to 7·0 *p*H by titration, then the litmus solution was added; this gave a very faint acid tint which showed any change of reaction quite easily, but no precipitate came down when steamed at 100° C. Even when the *p*H was brought to 7·4 some precipitate came down on steaming.

METHODS FOR THE INVESTIGATION OF THE FERMENTATIVE CHARACTERISTICS OF THE ORGANISMS.

When treatment is not mentioned, the sub-cultures were incubated at 30° C., and the results observed from day to day and tabulated.

Nitrate reduction test.

Nitrate broth was inoculated and reduction of nitrate to nitrite was tested at the end of 10, 20, and 30 days.

10 c.c. of water and 1 c.c. of nitrite test solution (α-naphthylamine sulphanilic acid) were taken in a test-tube and a loopful of the solution to be tested was introduced. In the presence of nitrite the liquid turned pink in a few minutes. In case of absence of nitrite the medium was tested by means of dyphenylamine and sulphuric acid; the development of a deep blue colour showed the presence of a nitrate. If the test proved to be negative, it was concluded that the nitrate had been completely reduced to ammonia or nitrogen.

Diastase Reaction.

Potato-plug cultures were directly examined for diastatic reaction. Four potato-plugs were inoculated at the same time and tested at the end of 15, 30, 45, and 60 days.

1 c.c. of potato-plug infected, and 1 c.c. of potato-plug control (uninoculated) were crushed each in 100 c.c. of distilled water in a mortar, the liquid poured into test-tubes, and a few drops of alcoholic iodine added to each; when decolorisation of iodine showed very strong diastatic reaction.

Pink colour	showed	strong reaction
Violet ,,	,,	moderate ,,
Bluish-violet ,,		trace of diastatic reaction
Blue colour ,,		absence of ,, ,,

N.B. In case of chromogenic growth, it was carefully scraped off first and the part of the underlying tissue which showed no coloration was taken for the test; otherwise the iodine would give misleading colour with the tested liquid.

Indol Production of Bacteria.

1 per cent. ordinary peptone-water solution was inoculated and tested for indol after 7, 14, 21, and 28 days.

Ehrlich test is the most satisfactory. The reagent is prepared by dissolving 4 gm. of paradimethylamino-benzaldehyde in 380 c.c. of alcohol and adding 80 c.c. of concentrated hydrochloric acid to it.

2 c.c. of the reagent was added to 2 c.c. of the liquid culture; a pink colour was formed in the presence of indol at the junction of the two liquids. When the reaction was feeble the ring appeared after 2–3 hours' standing. Addition of potassium persulphate is not necessary and gives misleading results on long standing.

For nitrite, diastase, and indol reaction and for gram-staining, chromogenic organisms were incubated at 37° C. They partly lost their power of producing pigment at 37° C. and the true colour reaction could be seen.

MICROSCOPIC EXAMINATION.

Examination of Living Bacteria.

The hanging drop method was used in order to determine motility, spore formation, and the arrangement of organisms.

A tiny loopful of the organism was taken in a loopful of water on a slide, covered with a cover-glass and sealed with paraffin on all four sides, so that any movement of the drop as a whole was prevented. When the slide thus prepared was heated to 37° C. the motile organisms moved about very briskly.

EXAMINATION OF STAINED BACTERIA.

48 hours' cultures on agar are usually the best for ordinary stained preparations. Films were prepared in the ordinary way for spore, Gram, and capsule staining.

Spore Staining.

Moeller's double stain method—as described by Eyre (6)—for staining spores gave most satisfactory results.

Gram's Method.

The following modification of Hucker's (7) method was found to be the most satisfactory, but for counter stain fuschsin solution gives better results than Bismarck Brown solution. The staining fluid is made up according to Hucker's original directions.

Preparation of Film and Fixing.

The film when dry was fixed by dropping absolute alcohol over it, draining and burning off the alcohol—temperature 60°–70° C. at the most.

Anilin gentian violet solution was filtered directly on to the cold slide and the stain drained off after 40 seconds.

Gram's iodine solution was then dropped on the film and drained off after 40 seconds.

The slide was de-stained by holding it in a slanting position and by pouring decolorising solution[1] drop by drop on the film until no more stain came off; it required from 10 to 15 seconds.

The slide was then well washed in water, and counterstained by flooding it with water and pouring four drops of fuchsin solution on it. This very dilute fuchsin was allowed to act for 30 seconds and washed off. Gram positive organisms then appeared bluish-violet and Gram negative reddish-brown.

This method yielded most consistent and satisfactory results. Consistent results could not be obtained by using alcohol or acidic alcohol as a decoloriser.

The addition of the anilin oil and xylol to the decolorising alcohol retards the action of the solution sufficiently to allow the Gram positive organisms to retain the stain, while the colour is removed from the Gram negative bacteria.

All the spores tried so far were Gram positive, and since the spores do not take up the gentian violet stain, but are coloured light pink by

[1] Aniline 2 parts, Xylol 1 part, 95 % Alcohol 60 parts.

the fuchsin counterstain, this method can also be used as a double stain for spores.

Capsule staining.

The following Johnes (8) method gave better results than Ribberts' (9) method.

The film when dried was covered with a 2 per cent. solution of gentian violet, heated until it steamed, then washed with water and moistened with 2 per cent. acetic acid for 6 to 10 seconds, and washed off with water. The capsules were seen most beautifully when examined in water.

Staining of Flagella.

18–24 hours' culture on moist agar, incubated at 30° C. usually proved satisfactory.

A drop of water was taken on a clean slide, the growth gently removed with a platinum loop and stirred very carefully into this drop of water. A fresh loop of water was taken on a clean part of this slide, and a loop of the dilution mixed with it. To this a loopful of 2 per cent. osmic acid solution was added very gently. A scrupulously clean cover-glass was taken; it was passed quickly through a Bunsen flame about five times. A tiny loopful of the above suspension was taken and drawn once across the cover-glass in a zigzag manner and allowed to dry in the air. The cover-glass carefully held by one corner, in cornet spring-clip forceps, is stained by the method of Casares-Gil, as described by Plimmer and Paine (10).

Measurement.

A thin film was spread out on the slide, dried, and fixed with a 10 per cent. formalin solution for 10 minutes, then treated with methyl-violet saturated aqueous solution for 10 minutes in the cold, and finally washed with water.

Organisms with an envelope of mucilage were not stained uniformly by methyl-violet solution, hence they did not give correct diameter. These capsulated organisms were best measured in a nigrosin preparation.

An emulsion of the culture was made in a drop of water on a slide and to this was added from three to four loopfuls of concentrated nigrosin solution. The edge of a second slide was dipped in this and then laid on a fresh slide, so that an even film was formed between them. The slides were then gently drawn apart and the film allowed to dry in the air. The measurement of micro-organisms was carried out by means of a stage micrometer in combination with an eyepiece micrometer.

About 100 organisms were measured every time, and the average result taken as the size of that particular organism. In every case 48 hours' growth on agar at 30° C. was taken.

THERMAL DEATH POINT.

Thermal death point is that temperature which, with certainty, kills a liquid suspension of the organisms in question after an exposure of exactly 10 minutes. A new method by which the thermal death point of spores as well as that of vegetative bacteria could be determined, was worked out.

A 48 hours' lentil broth culture incubated at 30° C. was taken, and the thermal death point determined in the following way[1]:

The broth culture is drawn into sterile capillary pipettes and the ends sealed. These pipettes are then immersed in a water bath for exactly 10 minutes, at a range of temperature differing by 2° from 48° to 70° C. After heating, the tubes are at once broken with sterile forceps and a small drop of the liquid is placed on the oven-dried surface of nutrient agar in a Petri dish which is then incubated at 30° C. One dish will suffice for inoculations from twelve pipettes, thus the effect of heating in the whole range from 48° to 70° will appear on one plate. At times no colonies are visible until the fifth or sixth day.

It is convenient to use two pipettes so that by the time one is ready to come out of the bath the other is at hand to go in for the next rise of temperature. The same pipette can be used over and over again for the same culture for one set of different temperatures.

ATTEMPTS TO DETERMINE THE THERMAL DEATH POINT OF SPORE-BEARING ORGANISMS.

Several experiments were tried in different *p*H tubes for different periods and at different temperatures, without much success. The following experiment gave fairly satisfactory results:

The spore-bearing organisms were inoculated in 5 c.c. of lentil broth and incubated at 25° C. for 18 hours. An equal quantity of fresh lentil broth was then added to each of the culture tubes and incubated again at 25° C. *B. mycoides* and *B. anthracoides* were found to be free from spores at the end of 3–4 hours; *B. mesentericus* at the end of 4–5 hours; and *B. subtilis* at the end of 5–6 hours when examined microscopically. Thermal death point was then determined as above, keeping the culture tubes in ice water at 8° C. to inhibit further growth during the experi-

[1] This method was devised by Professor S. G. Paine and has since been used very successfully in his laboratory.

ments. Several experiments were tried with each organism, out of which only four gave satisfactory results, all the others showed growth above 80° C. *B. mycoides* seems to give fairly constant results.

Thermal Death Point of Spores.

An oil bath is fitted up for temperatures between 90° C. and 130° C. It consists of a round copper vessel 12 × 8 cm., furnished with a rectangular wire-work at the top, to which the capillary tube is fixed by another piece of rather stiff copper wire, so as to ensure its complete immersion in the oil.

When determining thermal death point of spores the liquid culture is sealed up hermetically in a capillary tube. This prevents it from drying up, as the thermal death point determined under moist and dry conditions varies widely, and also, if one end is open, as soon as the capillary tube is inserted in the hot bath the liquid thread is pushed up in the capillary tube above the surface of the oil in the bath, owing to the expansion of the small quantity of air which is left in the free space at the sealed end.

Each tube is exposed in turn for 10 minutes to different fixed temperatures, varying by 5° C. between 80° C. and 130° C. The method of inoculation is the same as in the case of the vegetative forms, except that both the ends of the capillary tube are crushed open to deposit a drop of the suspension on to the solid medium.

Advantages of this Method over the Older Methods.

1. The tube being very thin, the liquid thread attains the temperature of the bath immediately, so that the organism is exposed to that particular temperature exactly for the whole period of immersion.
2. Also, being only a liquid thread, the whole portion of the liquid is heated uniformly.
3. The duplicate results are always found to be uniform in each case, the thickness of the glass of the pipette being almost the same in all cases.
4. In addition it is economical, for only one tube of broth and two tubes of solid medium are required for the whole process.

By the kindness of Dr St J. Brooks I was able to obtain the original cultures of all the species and the different varieties of those species, tested, from the National Collection of Type Cultures at the Lister Institute, London.

I desire to express my indebtedness to Dr S. G. Paine for his help and criticism.

BIBLIOGRAPHY.

(1) BALLAND (1912). (*Compt. rend.* 1897, pp. 125, 119.) *Dictionary of Applied Chemistry*—Thorpe, III, 311. (Longmans, Green & Co.)

(2) CHURCH (1912). Food grains of India 1886 and 1891. *Dictionary of Applied Chemistry*—Thorpe, III, 311. (Longmans, Green & Co.)

(3) KONIG (1912). *Dictionary of Applied Chemistry*—Thorpe, III, 311. (Longmans, Green & Co.)

(4) GILLESPIE, L. J. (1920). Colorimetric determination of Hydrogen Ion Concentration without Buffer Mixtures, with special reference to soils. *Soil Science*, IX, 115. Also *Journ. Ann. Chem. Soc.* XLII, 742.

(5) EYRE, J. W. H. (1902). *The Elements of Bacteriological Technique*, p. 171.

(6) —— (1913). *Ibid.* p. 107.

(7) HUCKER, G. J. (1921). A New Modification and Application of the Gram Stain. *Journal of Bacteriology*, VI, no. 4, 395.

(8) LEHMANN AND NEUMANN (1901). *Bacteriology*, II, text, p. 478.

(9) EYRE, J. W. H. (1913). *The Elements of Bacteriological Technique*, p. 101.

(10) PLIMMER, H. G. and PAINE, S. G. (1921). A New Method for the Staining of Bacterial Flagella. *The Journal of Pathology and Bacteriology*, XXIV.

BACCILLUS COLI COMMUNIS (86)[1].

Very short bacillus, size $0{\cdot}8$–$2{\cdot}3\mu \times 0{\cdot}5$–$0{\cdot}7\mu$, appearing like a large coccus, occurring in packets of 2 or 4. Feebly motile by means of 1 to 8 long wavy peritrichous flagella, which are very brittle. Gram negative, not capsulated, non-chromogenic, aerobic, and also facultative anaerobic. No spores observed.

Gelatine plate. Very minute, circular, translucent dots after 24 hours, which turn quite opaque after 3 days. Deeper colonies smaller, lenticular, yellowish. It does not liquefy gelatine.

Gelatine stab. Villous growth, with greyish-white surface growth in 48 hours.

Gelatine streak. Growth rather translucent with erose margin in 36 hours. Somewhat spreading, with greenish-yellow tint.

Agar plate. Opalescent circular dots with entire margin in 24 hours, turn yellowish in 3 days.

Agar streak. Whitish shining growth spreading over the slant with wavy smooth margin.

Broth. Moderately turbid, with some slimy deposit. Ring and pellicle absent.

Litmus milk. Acidic; white curd at the bottom, occupying $\frac{1}{6}$th of tube on 4th day. No digestion of casein is observed.

Potato culture. Dirty yellow growth.

Diastase reaction. Positive on 15th day.

Peptone water. Good growth, very turbid.

Indol reaction. Positive on 7th day.

Nitrate medium. Gas absent. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Feebly acid. Gas evolved on 8th day, ring present. Litmus bleached.

[1] The numbers quoted are the reference numbers of the cultures maintained in the National Collection of Type Cultures at the Lister Institute, London.

Sugar media:

Glucose. Acid; gas present.

Lactose. Acid; gas present.

Saccharose. No acid; no gas.

Thermal death point. 54°–56° C.

BACILLUS COLI COMMUNIOR (419).

Oval, short, and fat bacillus, appearing like big coccus, size 0·8–3·0 $\mu \times 0{\cdot}7$–0·9 μ, usually occurring in pairs. Feebly motile by means of 3 to 8 wavy peritrichous flagella which are very brittle. Gram negative, not capsulated, non-chromogenic, aerobic and also facultative anaerobic. No spores observed.

Gelatine plate. Very minute, circular, translucent dots after 24 hours, which grow slightly bigger and turn opaque on the 3rd day. Deeper colonies smaller, lenticular, and yellowish.

Gelatine stab. Echinate growth, surrounded by an irregular chain of small, translucent, spherical, colonies along the track of the needle in 48 hours.

Gelatine streak. Good growth in 24 hours, pale bluish-white at first, which turns dirty white, spreading laterally with undulate margin.

Agar plate. Smooth, shining, greyish-white small convex dots.

Agar streak. Moist, greyish-white growth; the growth rapidly spreads over the surface with undulate margin.

Broth. Very turbid. Pellicle and ring present.

Litmus milk. Acid; acid curds occupying $\frac{1}{6}$th of the tube on 4th day. No digestion of casein is observed.

Potato culture. Light cream growth.

Diastase reaction. Positive on 15th day.

Peptone water. Copious growth.

Indol reaction. Positive on 7th day.

Nitrate medium. Gas absent. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Feebly acid. Gas evolved on 7th day, ring present; litmus bleached.

Sugar media:

Glucose. Acid; copious gas evolved.

Lactose. Acid; copious gas evolved.

Saccharose. Acid; gas evolved.

Thermal death point. 54°–56° C.

BACILLUS COLI MUTABILIS (1419).

Oval, fat, short rods, appearing like coccus, size 0·8–1·5 $\mu \times 0{\cdot}7$–0·9 μ, frequently linked in pairs or short chains. Non-motile. Gram negative, not capsulated, non-chromogenic, aerobic, and also facultative anaerobic. No spores observed.

Gelatine plate. Raised, moist, opaque dots after 24 hours, which turn greyish-yellow on 3rd day. Deeper colonies pale brown, smaller.

Gelatine stab. Dirty greyish-white arborescent growth.

Gelatine streak. Good growth in 24 hours, moist greyish-white, with entire margin.

Agar plate. Opaque, shining, greyish-white colonies, very small, with entire margin.

Agar streak. Good growth, with undulated margin, very little tendency to spread.

Broth. Marked turbidity. Some sediment deposited. Ring and pellicle absent.

Litmus milk. Acid; coagulation positive on 2nd day; digestion of casein begins on 4th day; it is almost complete in 4 weeks.

Potato culture. Light yellow growth, changing to dirty yellow.

Diastase reaction. Positive on 15th day.

Peptone water. Good growth.

Indol reaction. Positive on 7th day.

Nitrate medium. Gas absent. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Acid, gas evolved on 5th day, ring present.

Sugar media:

Glucose. Acid; copious gas evolved.

Lactose. Acid; copious gas evolved.

Saccharose. No acid; no gas.

Thermal death point. 54°–56° C.

BACILLUS PRODIGIOSUS (1377).

Very short bacillus, with rather pointed ends, size 1·0–1·9 μ × 0·9–1·1 μ, occurring often in pairs, sometimes as threads. Actively motile by means of 4–6 or 8 peritrichous flagella. Gram negative, not capsulated, forms bright red to rich crimson pigment, oxygen seems to favour it, surface layer and surface colonies are always richer in hue, deep colonies are colourless; it is aerobic and also facultative anaerobic, in which case it loses its power of pigment production. No spores observed.

Gelatine plate. Small translucent grey colonies appear within 18 hours, and early liquefaction occurs. Colonies pink and irregular in outline, which soon fuse into one another, owing to rapid liquefaction.

Gelatine stab. Infundibuliform pink growth in 18 hours. Saccate in 54 hours. Stratiform on 4th day. Liquefaction with crimson colour on 5th day.

Gelatine streak. Pink growth in 18 hours, rapid liquefaction; whole turns crimson in 36 hours.

Agar plate. Minute pale yellow colonies round, with entire margin, in 18 hours. Elevated moist shining pink colonies, 1–3 mm. in size on 3rd day.

Agar streak. Yellow uniformly raised growth, with entire margin. It turns light pink on 3rd day, getting darker as its gets older.

Broth. Copious growth. Liquid yellow for some days, then it turns pink. Ring and pellicle absent.

Litmus milk. Slighly acid. Coagulation positive in 54 hours; digestion of casein begins on 4th day, completes in 3 weeks, except a pink dirty lump at the bottom.

Potato culture. Rose-red, moist, elevated growth, dark purple in 3 days.

Diastase reaction. Positive on 15th day.

Peptone water. Pink in 48 hours.

Indol reaction. Negative.

Nitrate medium. Gas absent. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Feebly acid. Gas absent. Litmus bleached on 8th day.

Sugar media:

Glucose. Strongly acid; gas present.

Lactose. No acid; no gas.

Saccharose. Acid; gas present.

Thermal death point. 52°–54° C.

BACILLUS VIOLACEUS (1539).

Thin rods with rounded ends, size 1·5–4·5 μ × 0·6–0·8 μ, occurring singly or in pairs. Active serpentine motion by means of 2–4–6 peritrichous flagella. Gram negative, not capsulated, forms brownish-white to violet pigment. Colour is first developed near the surface, later deeper in the liquid. Surface growth on gelatine is of very deep violet colour. It is aerobic and also facultative anaerobic, in which case it loses its power of pigment production. No spores observed.

Gelatine plate. Small yellow dots after 24 hours. Deeper colonies bigger and transparent. On 4th day light to violet in colour. On 7th day round saucer-shaped depression, due to liquefaction, with dark violet spot in the centre. Size 6·0 mm.

Gelatine stab. Filiform growth in 54 hours with crenate margin; napiform on 10th day with dark violet lobulated growth near the surface.

Gelatine streak. Violet sunken uniform growth, quite copious in 18 hours, with entire margin. Slow liquefaction in 54 hours, a violet deposit at the bottom.

Agar plate. Moist, shining, elevated yellowish dots in 24 hours, dirty buff, 2 mm. in size in 3 days.

Agar streak. Moist, raised, uniform growth, dirty white to violet along the streak. Margin lobar-lobulate.

Broth. Dirty grey copious growth. Ring and pellicle absent.

Litmus milk. Almost neutral. No coagulation, no digestion of casein. Milk turns deep violet, with a deeper ring at the surface in 5 weeks.

Potato culture. A mixture of violet and yellow moist shining growth.

Diastase reaction. Feeble on 30th day.

Peptone water. Good growth, pellicle at the surface on 5th day.

Indol reaction. Negative.

Nitrate medium. Gas absent; nitrate reduced to nitrite on 10th day.

Uschinsky solution. Feebly alkaline; gas absent; pellicle present. Violet after a fortnight.

Sugar media:

Glucose. No acid; no gas.

Lactose. No acid; no gas.

Saccharose. No acid; no gas.

Thermal death point. 48°–50° C.

PSEUDOMONAS FLUORESCENS LIQUEFACIENS (950).

Short slender rods with rounded ends. Size 1·5–3·8 μ × 0·4–0·6 μ, occurring usually isolated, often in pairs. Actively motile by means of single polar flagellum. Gram negative, not capsulated, forms yellowish-green fluorescent pigment which turns the medium yellow-brown. It is markedly aerobic, very scanty growth under anaerobic condition. No spores observed.

Gelatine plate. Yellowish round dots in 18 hours. Liquefaction begins very early. Area of liquefaction crateriform, size 5 mm. on 3rd day.

Gelatine stab. Crateriform growth with gas bubbles in 24 hours; later stratiform; no growth deeper in the tube. It is observed to be markedly aerobic.

Gelatine streak. Medium liquefied where streaked in 24 hours, leaving a hollow track behind. Light green growth collected at the base of the slope.

Agar plate. Yellowish round colonies with entire margin, umbonate, about 2 mm. in size on 4th day. The medium shows slightly greenish tint.

Agar streak. Dirty white uniform growth with entire margin, medium greenish-yellow, water of condensation full of gas bubbles.

Broth. Uniformly turbid. Gas bubbles at the surface. Ring and pellicle present. No fluorescence seen.

Litmus milk. Strongly alkaline in 18 hours. No coagulation. Digestion of casein begins on 3rd day, completes in 2 weeks.

Potato culture. Buckthorn-brown coloured growth.

Diastase reaction. Negative.

Peptone water. Good growth; ring and pellicle at the surface.

Indol reaction. Negative.

Nitrate medium. Copious gas evolved. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Alkaline. Gas absent; pellicle present; bluish-green fluorescence on 4th day.

Sugar media:

Glucose. Acid; gas absent; ring and pellicle present.

Lactose. No acid; no gas; ring present.

Saccharose. No acid; no gas; ring present.

Thermal death point. 52°–54° C.

PSEUDOMONAS FLUORESCENS NON-LIQUEFACIENS (912).

Short slender rods, rounded at ends, size 1·5–4·5 $\mu \times 0{\cdot}7$–0·9 μ, occurring singly or in pairs. Actively motile, usually by means of 1 polar flagellum, sometimes a bundle of 3–6 at one end. Gram negative, not capsulated, forms pale yellowish-green fluorescent pigment. Markedly aerobic, very scanty growth under anaerobic condition. No spores observed.

Gelatine plate. Opalescent circular dots in 18 hours, slight fluorescence seen round the colonies, which are 5–6 mm. on 4th day. No liquefaction.

Gelatine stab. Very slight growth, first filiform, then echinate. No growth deeper in the tube. Markedly aerobic.

Gelatine streak. Good growth in 24 hours. Dirty white, almost transparent, growth along the streak, with entire margin.

Agar plate. Round, opaque, white umbonate colonies, with entire margin, about 5 mm. on 4th day. Medium greenish in tint.

Agar streak. Translucent dirty white growth, with undulate margin. Medium shows slight greenish-yellow tint.

Broth. Good growth, no gas. Ring and pellicle present. No fluorescence.

Litmus milk. Strongly alkaline in 18 hours. No coagulation. No digestion of casein.

Potato culture. Fawn-coloured growth.

Diastase reaction. Negative.

Peptone water. Good growth; ring or pellicle absent.

Indol reaction. Negative.

Nitrate medium. Good growth. Gas absent. No reduction of nitrate is observed, even after 6 weeks.

Uschinsky solution. Alkaline. Gas absent; pellicle present. Greenish-yellow fluorescence on 3rd day.

Sugar media:

Glucose. Acid; no gas.

Lactose. No acid; no gas. Ring and pellicle present. Gets gradually bleached.

Saccharose. No acid; no gas. Ring present. Bluish fluorescence on 3rd day.

Thermal death point. 48°–50° C.

PSEUDOMONAS PYOCYANEUS (1540).

Very slender rods with rounded ends, size 1·5–3·8 $\mu \times 0{\cdot}3$–0·5 μ, occurring singly. Actively motile by means of single polar flagellum. Gram negative, capsulated, gives yellowish-green to dark bottle-green fluorescence. Aerobic, very scanty growth under anaerobic condition. No spores observed.

Gelatine plate. Granular saucer-shaped depression 1 mm. in size, in 18 hours. Medium shows beautifully green fluorescence. In 48 hours the liquefaction spreads widely and the colonies unite with each other.

Gelatine stab. Cylindrical growth in 36 hours. Infundibuliform in 54 hours. Stratiform on 10th day. Markedly aerobic.

Gelatine streak. Cream-coloured growth in 18 hours. Slow liquefaction along the streak.

Agar plate. Small, round yellowish-white translucent colonies in 18 hours, 4–6 mm. in size on 7th day. Yellowish-green fluorescence.

Agar streak. Uniform dirty greenish-yellow coloured moist growth along the streak. Margin erose, water of condensation full of gas bubbles in 18 hours. Medium turns green on 4th day.

Broth. Copious growth with yellowish-green fluorescence. Bubbles at the surface. Pellicle formed on 3rd day.

Litmus milk. Alkaline in 54 hours. No coagulation. Digestion of casein begins on 4th day. Complete on 12th day, finally leaving a green fluorescent liquid, and a small quantity of dirty green deposit at the bottom.

Potato culture. Sometimes yellow, sometimes green-coloured growth.

Diastase reaction. Negative.

Peptone water. Light green in 36 hours, dark green on 5th day, at times shows bluish fluorescence. It turns salmon pink after a fortnight.

Indol reaction. It is not possible to determine indol reaction from neutral peptone water as any acid, *e.g.* a drop of dilute hydrochloric acid, when added to the green liquid culture gives immediately a pink colour. When grown in acid peptone, no green colour is produced, and then tested for indol, it is found to be absent.

Nitrate medium. Copious gas evolved in 18 hours. Liquid turns pinkish-yellow on 15th day. Nitrate reduced to nitrite on 10th day. No trace of nitrate or nitrite left after 15 days, owing to complete reduction.

Uschinsky solution. Alkaline. Gas absent. Bluish-green ring and pellicle present in 18 hours. Yellowish-green fluorescence on 3rd day.

Sugar media:

Glucose. Acid on 5th day; no gas.

Lactose. No acid; no gas. Peacock-coloured on 3rd day; ring and pellicle present.

Saccharose. No acid; no gas. Peacock-coloured on 3rd day; ring and pellicle present.

Thermal death point. 52°–54° C.

BACILLUS PROTEUS VULGARIS (402).

Slender rods of varying sizes 1·5–6·0 μ × 0·4–0·6 μ, occurring often as long threads, also singly or in pairs. Actively motile, by means of many long peritrichous flagella. Gram negative, not capsulated, non-chromogenic, aerobic and also anaerobic. No spores observed.

Gelatine plate. Grey transparent depressions in 18 hours. These spread irregularly in 48 hours.

Gelatine stab. Greyish-white crateriform growth in 36 hours. Saccate in 54 hours.

Gelatine streak. Rapid liquefaction along the streak; white growth collected at the bottom.

Agar plate. Round, moist, glistening, translucent dots, with entire margin, 1 mm. in 48 hours. Deep colonies smaller in size, lenticular.

Agar streak. Greyish-white moist, glistening, transparent growth which spreads all over the surface with erose margin. Water of condensation quite turbid.

Broth. Uniformly turbid. Ring and pellicle absent. Dirty white deposit.

Litmus milk. Feebly acid. Coagulation positive on 3rd day. Digestion of casein begins on 4th day, complete in 3 weeks.

Potato culture. Dirty yellowish-grey or vinaceous buff-coloured growth.

Diastase reaction. Feeble on 30th day.

Peptone water. Copious growth.

Indol reaction. Positive on 7th day.

Uschinsky solution. Slightly acid; gas absent.

Nitrate medium. Gas absent; nitrate reduced to nitrite on 10th day.

Sugar media:

Glucose. Acid; gas evolved in 18 hours.

Lactose. No acid; no gas; litmus bleached.

Saccharose. Acid; gas evolved on 8th day.

Thermal death point. 50°–52° C.

BACILLUS PROTEUS MIRABILIS (403).

Short, thick rods, with rounded ends; size 0·8–3·0 μ × 0·7–0·9 μ, occurring often as threads in matted arrangement, single or in pairs. Actively motile, by means of many peritrichous flagella. Gram negative, not capsulated, non-chromogenic, aerobic, and also anaerobic. No spores observed.

Gelatine plate. White, circular, or oval, finely granular colonies, in 18 hours, spreading irregularly with wavy outline.

Gelatine stab. A dense white layer of growth circular in outline on the surface of the medium. The liquefaction is not so rapid as in the case of *Proteus vulgaris.* Crateriform in 36 hours, stratiform on 4th day.

Gelatine streak. White growth in 24 hours, slow liquefaction along the streak.

Agar plate. Small, round, irregular, yellowish colonies, in 24 hours. Each colony surrounded by 4–5 translucent zones on 5th day, with pale yellow original colony in the centre. In some cases 9 zones could be distinctly counted on the 9th day.

Agar streak. Yellowish-white moist glistening growth spreading very rapidly, margin erose. Water of condensation quite turbid.

Broth. Uniformly turbid copious growth. Ring and pellicle absent. White deposit.

Litmus milk. Feebly acid on 2nd day. Turns feebly alkaline on 3rd day. Coagulation positive on 3rd day. Digestion of casein begins on 4th day, completes in 3 weeks.

Potato culture. Fawn-coloured growth.

Diastase reaction. Feeble on 30th day.

Peptone water. Good growth.

Indol reaction. Positive on 14th day.

Nitrate medium. Gas absent. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Acid; gas absent; ring present.

Sugar media:

Glucose. Acid; gas evolved in 18 hours.

Lactose. No acid; no gas.

Saccharose. Acid; gas evolved on 8th day.

Thermal death point. 52°–54° C.

PROTEUS (ZOPFIUS) ZENKERI (405).

Slender rods of varying size 1·5–6·0 $\mu \times 0{\cdot}7$–0·9 μ, occurring as long threads or as isolated short rods. Actively motile by means of many peritrichous flagella. Gram negative, not capsulated, non-chromogenic, aerobic and also facultative anaerobic. No spores observed.

Gelatine plate. Delicate greyish-white colonies about 7 mm. in diameter in 18 hours; they look like little pieces of fluff which can easily be mistaken for moulds. The mycelium-like branching spreads wide in 48 hours.

Gelatine stab. A fine-branched growth, which spreads from the track of the needle parallel to the surface. Branches are longest near the surface, and become short deeper in the tube. No liquefaction, no putrid smell as in the case of *Proteus vulgaris* and *mirabilis.*

Gelatine streak. Greyish-white transparent growth with thick streak in the centre in 48 hours. Later fine-branched growth.

Agar plate. Greyish-white irregular colonies, surrounded by a thin, transparent zone in 24 hours, about 6 mm. in diameter. Some colonies mycelium-like, with exceedingly tangled branching, and pale yellow on 4th day.

Agar streak. Greyish-white, transparent, shining thin growth, which rapidly spreads over the whole surface, with lacerate margin.

Broth. Good growth. Ring and pellicle absent.

Litmus milk. Alkaline on 3rd day. No coagulation. No digestion of casein. Milk decolorised in 3 weeks.

Potato culture. Yellowish cream-coloured growth, changing to fawn.

Diastase reaction. Negative.

Peptone water. Good growth.

Indol reaction. Positive on 14th day.

Nitrate medium. Gas absent. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Acid; gas absent.

Sugar media:

Glucose. Acid; gas evolved in 18 hours.

Lactose. No acid; no gas; pellicle present; litmus bleached.

Saccharose. Acid; gas evolved on 5th day.

Thermal death point. 52°–54° C.

SARCINA LUTEA (611).

Almost spherical in appearance, fairly uniform in size, $1{\cdot}5\mu \times 1{\cdot}1\mu$, occurring in packets of 8 or a multiple of 8, in pairs, in fours, or in cubes, which present the characteristic appearance of corded bales of cotton. Non-motile, flagella absent. Gram negative, not capsulated, forms sulphur-yellow pigment; aerobic and also facultative anaerobic. No spores observed.

Gelatine plate. Sulphur-yellow, very fine raised dots on 3rd day; colonies a little sunk in on 5th day. Deeper colonies still smaller. The whole plate looks as if sprinkled over with mustard powder. Gelatine is liquefied on 7th day, the form of the colonies being disturbed, and broken up into granular structure, some colonies float about intact.

Gelatine stab. Pale yellow filiform growth in 36 hours, with a round depression at the surface, due to slow liquefaction; crateriform on 3rd day, stratiform on 7th day.

Gelatine streak. Pale yellow-coloured growth, very slow liquefaction in 18 hours where streaked; complete liquefaction in 36 hours, with a yellow deposit at the bottom, and yellow pellicle on the surface.

Agar plate. Pale yellow, moist, shining pulvinate round colonies, with even margin, 1–2 mm. in 36 hours; sulphur-yellow capitate, colonies 3–4 mm. on 4th day; lemon-yellow, 6 mm. on 7th day; umbonate on 14th day. Deeper colonies yellow, small, 2 mm. starlike on 7th day.

Agar streak. Sulphur-yellow, luxuriant, moist, shining growth, restricted to the streak; water of condensation clear, with light yellow deposit at the bottom in 48 hours; raised wax-yellow growth with wavy margin spreading on 3rd day; margin auriculate on 5th day.

Broth. Moderate growth with good quantity of yellowish-white deposit at the bottom; liquid almost clear, ring and pellicle absent.

Litmus milk. It turns alkaline on 3rd day; coagulates on 4th day; digestion of casein begins on 5th day and completes on 8th day, and the liquid turns yellow; the blue colour reappears on 10th day.

Potato culture. Strontian yellow, copious raised growth in 24 hours, restricted to the streak. The growth spreads slightly with erose margin on 3rd day.

Diastase reaction. Positive on 30th day.

Peptone water. Liquid, clear, with light yellow deposit in 36 hours.

Indol reaction. Negative.

Nitrate medium. Gas absent, liquid clear, good quantity of yellow deposited in 36 hours. No reduction of nitrate is observed, even after 6 weeks.

Uschinsky solution. Gas absent; ring present on 4th day; it turns alkaline on 6th day. Yellow deposited at the bottom.

Sugar media:

Glucose. No acid, no gas; greenish-yellow deposit on 5th day, ring present on 8th day, alkaline on 8th day.

Lactose. No acid, no gas; greenish-yellow deposit on 5th day; alkaline on 8th day; ring present on 10th day.

Saccharose. No acid, no gas; greenish-yellow deposit on 5th day; ring present on 8th day; alkaline on 8th day.

Thermal death point. 64°–66° C.

SARCINA FLAVA (196).

Size 1·6 μ × 1·1 μ, occurring usually in packets grouped together irregularly, occasionally singly isolated. Forms amber-yellow pigment.

Litmus milk. It turns alkaline on 5th day. No other change is observed till 7th day when it coagulates; digestion begins on 8th day and completes on 18th day.

Diastase reaction. Strong positive on 15th day. *Sarcina flava* resembles *Sarcina lutea* in every other respect.

SARCINA MILK (633).

Size 1·1 μ × 0·8 μ. Forms citron-yellow pigment.

Litmus milk. It turns slightly alkaline on 5th day. No other change is observed.

Diastase reaction. Positive on 45th day. *Sarcina milk* resembles *Sarcina lutea* in every other respect.

SARCINA AURANTIACA (952), (01346).

Round spheres, fairly uniform in size, with average diameter of 0·9 μ, occurring in packets of 3, 2, 4, or more, or single. Forms ochraceous-orange pigment.

Litmus milk. Alkaline in reaction; coagulation on 3rd day; digestion of casein begins on 4th day, completes on 8th day; with copious orange-red deposit at bottom.

Diastase reaction. Positive on 30th day.

Peptone water. Liquid cloudy, dirty yellow deposit in 48 hours; liquid full of orange-yellow particles; ring and pellicle present on 4th day.

Nitrate medium. Gas absent; liquid cloudy in 36 hours, with some dirty yellow deposit, ring present on 6th day; orange-yellow pellicle on 8th day.

Uschinsky solution. Gas absent; very scanty growth; alkaline on 12th day.

Sugar media:

Glucose. Acid on 3rd day, gas absent; some deposit at bottom.

Lactose. Acid on 3rd day; gas absent; some deposit at the bottom; ring present on 8th day; pellicle present on 12th day.

Saccharose. Acid on 3rd day, gas absent; some deposit at the bottom; ring present on 8th day; pellicle present on 12th day.

Thermal death point. 56°–58° C.

Sarcina aurantiaca resembles *Sarcina lutea* in every other respect.

BACILLUS ANTHRACOIDES (945).

Thick rods with square ends, size 3·0–10·5 μ × 1·4–1·7 μ, occurring in chains, single, or in pairs. Sluggishly motile by means of 2–8 peritrichous flagella which are very brittle. Gram positive, capsulated, non-chromogenic, aerobic, and also anaerobic. It forms oval spores size 1·8 μ × 0·8 μ.

Gelatine plate. Fine translucent white dots in 18 hours; big grumose colonies in 48 hours, perfectly round, white, deeply sunken, due to slow liquefaction under the colonies. Yellowish, moruloid, perfectly round, big colonies, about 20 mm. in diameter on 5th day. The size is about 25 mm. on 7th day. No liquefaction of the surrounding medium.

Gelatine stab. Crateriform growth in 18 hours, saccate in 48 hours, cloudy and granular in appearance.

Gelatine streak. Liquefaction under the streak in 18 hours; white growth with a pellicle on the surface of liquid gelatine in 48 hours.

Agar plate. Small opaque white round pulvinate colonies in 24 hours. Umbonate with undulate margin about 5 mm. in size on 5th day.

Agar streak. Greyish-white growth with undulate margin. The growth is raised and appears as if innumerable tiny air bubbles were lying beneath the surface of the growth.

Broth. Turbid with thin pellicle at the surface, which sinks to the bottom on shaking.

Litmus milk. Feebly alkaline; coagulation positive on third day; digestion of casein begins on 4th day, completes in 8 days.

Potato culture. Dirty white shining growth, turns yellowish-cream after a week.

Diastase reaction. Feeble on 30th day.

Peptone water. Turbid in 18 hours. Ring and pellicle present in 24 hours.

Indol reaction. Positive on 7th day.

Nitrate medium. Gas present on 5th day. Ring and pellicle present in 24 hours. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Alkaline; gas absent; slight deposit on 3rd day.

Sugar media:

Glucose. Strongly acid; gas absent; ring and pellicle present; litmus bleached.

Lactose. No acid; no gas; ring and pellicle present.

Saccharose. Strongly acid; gas absent; litmus bleached.

Thermal death points:

Spore form. 90°–95° C. Vegetative form. 66°–68° C.

BACILLUS MYCOIDES (926).

Rods with flat ends, size 2·3–6·0 $\mu \times 0{\cdot}9$–1·2 μ, arranged in long chains or threads. Feebly motile by means of 8 or more peritrichous flagella. Gram positive, capsulated, non-chromogenic. Aerobic and also anaerobic. It forms oval spores, size 1·8 $\mu \times 0{\cdot}9\,\mu$.

Gelatine plate. White, big, myceloid colonies in 18 hours, 10 mm. in diameter. Rapid liquefaction under the colonies in 48 hours. The whole plate gets covered up with network on the 5th day. No liquefaction of the surrounding medium.

Gelatine stab. Crateriform growth in 18 hours, characterised by delicate little hair-like growth of uniform length, growing outward parallel to the surface along the stab canal in 40 hours. There is a thick white film upon the surface of the liquid gelatine.

Gelatine streak. Rapid liquefaction in 18 hours; the white band of growth remains adhering to the side of the tube.

Agar plate. Circles of hair-like growth in 18 hours; good filamentous colonies in 48 hours, about 3–5 mm. in size. On 5th day rhizoid in appearance, with filamentous network to the diameter of about 50 mm. round each colony.

Agar streak. Greyish-white, moist, shining rhizoid growth, showing abundant branching which covers the whole surface in 3 days.

Broth. Turbid. Later a flocculent precipitate is formed and the liquid clears up. Pellicle present which drops to the bottom on shaking.

Litmus milk. Alkaline; coagulation positive on 3rd day; digestion of casein begins on 4th day; completes in 3 weeks.

Potato culture. Light pink or dull white slightly elevated growth.

Diastase reaction. Positive on 15th day.

Peptone water. Colloidal fluffy growth at the bottom of the tube in 18 hours.

Indol reaction. Positive on 14th day.

Nitrate medium. Gas present on 7th day; pellicle present. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Alkaline; gas absent.

Sugar media:

Glucose. Strongly acid; gas absent, flocculent growth floating about in the liquid.

Lactose. No acid; no gas; flocculent growth floating about in the liquid.

Saccharose. Acid; gas absent; flocculent growth floating about in the liquid.

Thermal death point:

Spore form. 85°–90° C. Vegetative form. 58°–60° C.

BACILLUS SUBTILIS (85).

Short rods with rounded ends, size $2{\cdot}3$–$5{\cdot}3\,\mu \times 0{\cdot}6$–$1{\cdot}0\,\mu$, occurring in chains or long threads. Actively motile by means of many peritrichous flagella. Gram positive, not capsulated, non-chromogenic. Aerobic and also anaerobic. It forms oval spores, size $1{\cdot}6\,\mu \times 0{\cdot}8\,\mu$.

Gelatine plate. Colonies in 48 hours, sunken due to liquefaction under the colonies. On 5th day about 12 mm. in diameter; 32 mm. in diameter on 7th day. It liquefies gelatine only under the colonies. No liquefaction of surrounding medium.

Gelatine stab. Crateriform growth in 36 hours. Saccate on 3rd day. Thick white pellicle at the surface.

Gelatine streak. Liquefaction along the streak. White growth. Liquid gelatine covered with a film on 5th day.

Agar plate. Some colonies with a dense centre and very transparent irregular branches, rhizoid in appearance, about 5 mm. in 48 hours. Others very irregular and much raised above the surface, very slimy in appearance. Deeper colonies very irregular and translucent.

Agar streak. Moist, opaque, white, thick growth, with entire margin; as the growth becomes older, its surface becomes dry and wrinkled up, especially at the bottom of the slope.

Broth. Uniformly cloudy. Wrinkled, dirty white thick pellicle at the surface, which does not drop to the bottom of the tube on shaking.

Litmus milk. Feebly alkaline; coagulation positive on 3rd day; digestion of casein begins on 4th day, completes in 8 days; pellicle present.

Potato culture. Dirty white growth; dirty grey wrinkled skin covers the whole plug on 2nd day.

Diastase reaction. Positive on 15th day.

Peptone water. Good growth. Pellicle present.

Indol reaction. Negative.

Nitrate medium. Gas absent. Pellicle present in 48 hours. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Strongly alkaline. Gas absent. Pellicle and ring present. Completely decolorised on 5th day. Blue colour reappears on 14th day.

Sugar media:

Glucose. Acid in 48 hours. Pellicle present. Bleached on 7th day. Gas evolved on 12th day.

Lactose. No acid. No gas. Pellicle present.

Saccharose. Acid on 3rd day. Pellicle present. Bleached on 7th day. Gas evolved on 12th day.

Thermal death point:

Spore form. 110°–115° C. Vegetative form. ?

BACILLUS MEGATHERIUM (654).

Very long and fat rods with rounded ends. Size 3·0–13·5 $\mu \times 1{\cdot}7$–2·2 μ, occurring in pairs, in chains, or single; when in pairs not in a straight line but like the figure 7. Sluggishly motile by means of many peritrichous flagella. Gram positive, not capsulated, non-chromogenic. Aerobic, very scanty growth under anaerobic condition. It forms oval spores, size 1·5 $\mu \times 1{\cdot}8\,\mu$. Almost invariably 2 spores were seen in each cell, placed end to end.

One to two hours' growth showed at 37° C. involution forms which were very long and fusiform and full of vacuoles.

In hanging drop, *B. megatherium* was seen containing minute discrete and highly refractile granules. When this organism was stained by Moeller's method, various granules in the cell were observed to stain differently from the substance of the cell-body, for example, red against a blue background. The granules were sometimes scattered through the cell substance, sometimes massed at either pole, where they constitute the polar granules.

These Moeller staining granules increase in number gradually and aggregate into two special masses, which dispose themselves one at each end of the cell and form spores; when the spores are fully formed the whole of the bacterium is absorbed and the spores are set free.

Gelatine plate. Round yellow dots in 18 hours. The colonies sink down in 48 hours owing to liquefaction. Big grumose colonies on 5th day, 10 mm. in diameter, covered with a wrinkled pellicle. No liquefaction of the surrounding medium.

Gelatine stab. Very cloudy crateriform growth in 18 hours; stratiform on 7th day; markedly aerobic.

Gelatine streak. Liquefaction along the streak in 18 hours; dirty grey-coloured growth.

Agar plate. White opaque tiny dots in 18 hours; round, white, moist shining, pulvinate colonies in 48 hours, with entire margin, about 2 mm. in size. Almost capitate on 5th day, about 12 mm. in diameter. The medium and growth coffee colour after a fortnight.

Agar streak. Cream, moist, shining, growth with undulate margin. Water of condensation turbid. Growth dirty grey colour on 4th day, turns darker as it grows older, finally medium and growth turn coffee colour.

Broth. Fairly good growth. Ring and pellicle absent.

Litmus milk. Feebly alkaline on 2nd day. No coagulation. Milk begins clearing up at the surface on 5th day. The liquid at surface pink, at the centre clear yellow, and at the bottom light blue, with some deposit on 10th day.

Potato culture. Cream, moist, shining growth, which turns dark brown gradually.

Diastase reaction. Positive on 30th day.

Peptone water. Good growth; dirty deposit on 3rd day.

Indol reaction. Positive on 28th day.

Nitrate medium. Gas absent; good growth. No reduction of nitrate is observed even after 6 weeks.

Uschinsky solution. Alkaline; gas absent.

Sugar media:

Glucose. Acid in 48 hours; gas absent.

Lactose. Acid in 48 hours; gas absent.

Saccharose. Acid on 7th day; gas absent.

Thermal death point:

Spore form. 100°–105° C. Vegetative form. 72°–74° C.

BACILLUS MESENTERICUS (656).

Slender rods with rounded ends almost uniform in size, 2·3–4·5 $\mu \times 0{\cdot}8$–1·1 μ, occurring in long chains, rarely single. Actively motile by means of many peritrichous flagella. Gram positive, not capsulated, non-chromogenic. Aerobic and also anaerobic. It forms almost round spores, size 1·2 $\mu \times 1{\cdot}0\,\mu$.

Gelatine plate. Tiny white translucent round dots with entire margin in 18 hours. On the 5th day the colonies are grumose and sunken, owing to liquefaction under the colonies, about 7 mm. in size. No liquefaction of the surrounding medium.

Gelatine stab. Transparent infundibuliform growth in 18 hours, crateriform on 5th day, pellicle present.

Gelatine streak. Liquefaction along the streak in 18 hours; liquid gelatine covered with white granular film.

Agar plate. Irregular, very tiny dots in 18 hours; veil-like transparent irregular growth with the original opaque colonies in the centre in 48 hours. Colonies 12 mm. in size covered up with wrinkled, dried up, greyish-white skin on 5th day.

Agar streak. Grey, dry, wrinkled growth with erose margin, pellicle on water of condensation.

Broth. Good turbidity. Ring and pellicle present. The film does not drop to the bottom of the tube on shaking.

Litmus milk. Feebly alkaline; coagulation positive on 3rd day; digestion of casein begins on 4th day, completes in 8 days.

Potato culture. Meshwork of much elevated, dull grey coloured growth in 36 hours, which curls up and turns yellow on 3rd day.

Diastase reaction. Positive on 15th day.

Peptone water. Good growth; film formed in 48 hours which does not sink on shaking.

Indol reaction. Negative.

Nitrate medium. Gas absent. Thick film and ring in 48 hours. The film does not sink on shaking. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Strongly alkaline; gas absent.

Sugar media:

Glucose. Strongly acid. Gas absent. Ring and pellicle present. Finally bleached.

Lactose. No acid. No gas. Ring and pellicle present.

Saccharose. Acid. Gas absent. Bleached on 4th day.

Thermal death point:

Spore form. 105°–110° C. Vegetative form. 70°–72° C.

BACILLUS CAROTOVORUS (386).

Short oval rods with rounded ends, size 1·1–2·3 $\mu \times 0{\cdot}7$–0·9 μ, occurring singly or in chains. Actively motile by means of 3 to 4 peritrichous flagella. Gram negative, not capsulated, non-chromogenic. Aerobic and also facultative anaerobic. No spores observed.

Gelatine plate. Translucent granular perfectly round tiny colonies, depressed in 24 hours, margin entire. Granular saucer-shaped colonies, surrounded by liquid gelatine on 3rd day.

Gelatine stab. Granular filiform growth in 24 hours, infundibuliform in 48 hours; saccate on 4th day, growth collected at the bottom. Complete liquefaction on 8th day, with copious yellowish-white granular deposit.

Gelatine streak. Liquefaction along the streak, white growth collected at the bottom of the slope in 24 hours. Liquefaction proceeds rapidly in 48 hours. Complete liquefaction on 7th day, with yellowish-white granular deposit.

Agar plate. Translucent moist shiny tiny colonies in 24 hours. Grayish-white with entire margin about 3 mm. in size on 3rd day. Some colonies throw out branches in two directions presenting peculiar appearance.

Agar streak. White translucent moist shiny growth in 24 hours, spreading over the slope on 3rd day, white granular deposit in the water of condensation.

Broth. Moderately turbid in 24 hours. White deposit at the bottom, thin pellicle present on 4th day.

Litmus milk. Acid on 2nd day. Litmus bleached on 4th day, dirty white deposit. Coagulation positive on 5th day; digestion of casein begins on 6th day, which is not complete, yellowish-white watery curd is seen even after 3 months.

Potato culture. White moist shiny growth in 24 hours. Gas bubbles are evolved on 2nd day. Growth spreads over the whole plug on 3rd day.

Diastase reaction. Strong positive on 15th day.

Peptone water. Moderately turbid in 24 hours. Very turbid on 3rd day.

Indol reaction. Negative.

Nitrate medium. Gas absent. Very turbid in 24 hours. Thin pellicle present on 7th day. Nitrate is reduced to nitrite on 10th day.

Uschinsky solution. Gas absent. Very turbid and alkaline on 3rd day. Lower half of the liquid bleached on 4th day. Thick copious deposit with ring at the surface on 8th day.

Sugar media:

Glucose. Acid; gas evolved in 24 hours. Litmus bleached on 3rd day. Copious deposit on 9th day.

Lactose. Acid; gas evolved in 24 hours. Litmus bleached on 3rd day. Copious deposit on 9th day.

Saccharose. Acid; gas evolved in 24 hours. Litmus bleached on 3rd day. Copious deposit on 9th day.

Thermal death point. 50°–52° C.

BACILLUS SOLANISAPRUS (385).

Rods with rounded ends, size 1·5–3·0 $\mu \times 0{\cdot}5$–0·7 μ, occurring singly or in pairs. Actively motile by means of 5–8 peritrichous flagella. Gram negative, not capsulated, non-chromogenic. Aerobic and also facultative anaerobic. No spores observed.

Gelatine plate. Tiny translucent dots in 48 hours. Bluish-white depressed round or oval colonies on 4th day with entire margin, cup-shaped on 5th day due to liquefaction.

Gelatine stab. White filiform growth in 24 hours; crateriform on 9th day; stratiform on 11th day.

Gelatine streak. White translucent growth with erose margin where streaked in 24 hours. Very slight depression on 3rd day. Slight liquefaction where streaked on 7th day, margin lacerate. Complete liquefaction on 11th day, dirty yellowish-white deposit at the bottom.

Agar plate. Moist white shiny translucent dots in 24 hours. Opaque granular raised tiny colonies on 4th day, rather irregular in outline.

Agar streak. Thin white translucent growth in 24 hours. Shining opalescent growth spreading over the slope on 3rd day, dirty white on 7th day.

Broth. Very turbid in 24 hours; fine deposit on 4th day.

Litmus milk. Acid on 3rd day. Litmus bleached on 4th day. Coagulation positive on 6th day; digestion of casein begins on 8th day, which is not complete, white watery curd is seen even after 3 months.

Potato culture. Moist white growth in 24 hours. Pale cream on 4th day which changes to dirty white.

Diastase reaction. Positive on 15th day.

Peptone water. Moderately turbid in 24 hours. Good turbidity in 48 hours.

Indol reaction. Negative.

Nitrate medium. Gas absent. Very turbid in 24 hours. Thin pellicle present on 6th day. Nitrate is reduced to nitrite on 10th day.

Uschinsky solution. Gas absent. Moderately turbid in 24 hours. Alkaline on 3rd day. Bleached on 5th day, ring present. Thick viscid deposit on 10th day.

Sugar media:

Glucose. Acid; gas evolved in 24 hours. Pink ring at surface, rest bleached to yellow liquid on 4th day. Some dirty deposit in 3 weeks.

Lactose. Acid; gas evolved in 24 hours. Pink ring at surface, rest bleached to yellow liquid on 4th day. Some dirty deposit in 3 weeks.

Saccharose. Acid; gas evolved in 24 hours. Pink ring at the surface, rest bleached to yellow liquid on 4th day. Some dirty deposit in 3 weeks.

Thermal death point. 48°–50° C.

BACILLUS PHYTOPHTHORUS (Appel).

Short rods with rounded ends, size 0·9–1·5 μ × 0·5–0·7 μ, occurring singly or in pairs. Actively motile by means of 5–10 long wavy peritrichous flagella. Gram negative, not capsulated, non-chromogenic. Aerobic and also facultative anaerobic. No spores observed.

Gelatine plate. Translucent bluish-white dots in 24 hours. White circular depressed colonies with entire margin about 3 mm. in size in 48 hours, 6–7 mm. on 5th day, liquefaction under the colonies on 7th day.

Gelatine stab. Translucent filiform growth in 24 hours, remains filiform for 4 days. Crateriform on 9th day.

Gelatine streak. White translucent growth where streaked in 24 hours, margin erose, and slight depression in 48 hours. Slight liquefaction where streaked on 5th

day, margin fimbriate. Complete liquefaction on 8th day, with dirty yellowish-white deposit.

Agar plate. Tiny round translucent bluish dots in 24 hours. Round moist shiny colonies about 1–2 mm. in diamter on 3rd day; about 2–3 mm. on 5th day.

Agar streak. White translucent growth in 24 hours, spreading over the slope on 3rd day. White granular deposit in the water of condensation.

Broth. Very little turbidity in 24 hours. Very fine sediment deposited on 9th day.

Litmus milk. Acid on 3rd day. Litmus bleached on 4th day. Coagulation positive on 5th day. Digestion of casein begins on 6th day, which is not complete, pink watery curd is seen even after 3 months.

Potato culture. Moist white growth in 24 hours. Yellowish-white on 4th day.

Diastase reaction. Positive on 15th day.

Peptone water. Slightly turbid in 24 hours. Good turbidity on 3rd day.

Indol reaction. Negative.

Nitrate medium. Gas absent; very turbid in 24 hours. Thin pellicle on 7th day. Nitrate is reduced to nitrite on 10th day.

Uschinsky solution. Gas absent. Moderately turbid in 24 hours. Alkaline on 7th day. Completely bleached on 12th day. Ring present.

Sugar media:

Glucose. Acid. A tiny air bubble evolved on 4th day, which disappears on 10th day.

Lactose. Acid. Ring on 3rd day. A tiny air bubble evolved on 4th day, which disappears on 10th day.

Saccharose. Acid. Some gas evolved in 24 hours which disappears on 14th day.

Thermal death point. 48°–50° C.

PSEUDOMONAS CAMPESTRIS (388).

Short rods with rounded ends slightly broader at one end than at the other, size 1·1–2·0 μ ×0·5–0·7 μ, occurring singly. Actively motile by means of one long polar flagellum. Gram negative, not capsulated. It forms pale to deep yellow pigment. Markedly aerobic, very scanty growth under anaerobic condition. No spores observed.

Gelatine plate. Pale yellow, moist, shiny colonies, round with entire margin 2–3 mm. in diameter on 3rd day. Colonies turn distinctly yellow on 5th day, some of which are irregular in outline.

Gelatine stab. Pale yellow filiform growth on 3rd day. There is no liquefaction.

Gelatine streak. Yellow, moist, shiny, scanty growth in 36 hours. Chrome-yellow growth on 4th day. There is no liquefaction.

Agar plate. Pale yellow moist shining dots in 48 hours. Dirty yellow, round colonies about 4–5 mm. in diameter on 4th day.

Agar streak. Yellow scanty growth with even margin in 36 hours. Moist shining sulphur-yellow, good growth on 3rd day, margin undulate.

Broth. Moderately turbid in 24 hours; good turbidity on 3rd day. Liquid turns pale yellow on 4th day.

Litmus milk. Alkaline on 3rd day, decolorised on 7th day, blue colour reappears on 15th day. No coagulation, no digestion of casein. Slight yellow deposit at the bottom after 3 weeks.

Potato culture. Light yellow shining growth along the streak in 24 hours, deep yellow on 3rd day.

	Spores	Aerobic	Anaerobic	Liquefaction gelatine	Glucose: Acid	Glucose: Gas	Lactose: Acid	Lactose: Gas	Saccharose: Acid	Saccharose: Gas	Nitrate medium: Nitrite	Nitrate medium: Gas	Diastase on potato	Uschinsky solution: Reaction	Uschinsky solution: Gas	Litmus milk: Coagulation	Litmus milk: Digestion
I. B. coli communis	−	+	+	−	+	+	+	+	−	−	+	−	+	Acid	+	+	−
II. B. coli communior	−	+	+	−	+	+	+	+	+	+	+	−	+	Acid	+	+	−
III. B. coli mutabilis	−	+	+	−	+	+	+	+	−	−	+	−	+	Acid	+	+	+
IV. B. prodigiosus	−	+	+	+	+	+	−	−	+	+	+	−	+	Acid	−	+	+
V. B. violaceus	−	+	+	+	−	−	−	−	−	−	+	−	Feeble	Alk.	−	−	−
VI. Ps. fluorescens liquefaciens	−	+	÷	+	+	−	−	−	−	−	+	+	−	Alk.	−	−	+
VII. Ps. fluor. non-liquefaciens	−	+	÷	−	+	−	−	−	−	−	−	−	−	Alk.	−	−	−
VIII. Ps. pyocyaneus	−	+	÷	+	+	−	−	−	−	−	+	+	−	Alk.	−	−	+
IX. Proteus vulgaris	−	+	+	+	+	+	−	−	+	+	+	−	Feeble	Neutral	−	+	+
X. Proteus mirabilis	−	+	+	+	+	+	−	−	+	+	+	−	Feeble	Acid	−	+	+
XI. Prot. (Zopfius) Zenkeri	−	+	+	−	+	+	−	−	+	+	+	−	−	Acid	−	−	−
XII. Sarcina lutea	−	+	+	+	−	−	−	−	−	−	−	−	+	Alk.	−	+	+
XIII. Sarcina flava	−	+	+	+	−	−	−	−	−	−	−	−	+	Alk.	−	+	+
XIV. Sarcina (Milk sp.)	−	+	+	+	−	−	−	−	−	−	−	−	+	Alk.	−	−	−
XV. Sarcina aurantiaca	−	+	+	+	+	−	+	−	+	−	−	−	+	Alk.	−	+	+
XVI. B. anthracoides	+	+	+	+	+	−	−	−	+	−	+	+	Feeble	Alk.	−	+	+
XVII. B. mycoides	+	+	+	+	+	−	−	−	+	−	+	+	+	Alk.	−	+	+
XVIII. B. subtilis	+	+	+	+	+	+	−	−	+	+	+	−	+	Alk.	−	+	+
XIX. B. megatherium	+	+	÷	+	+	−	+	−	+	−	−	−	+	Alk.	−	+	+
XX. B. mesenterius	+	+	+	+	+	−	−	−	+	−	+	−	+	Alk.	−	+	+
XXI. B. carotovorus	−	+	+	+	+	+	+	+	+	+	+	−	+	Alk.	−	+	+
XXII. B. solanisaprus (385) (Washington)	−	+	+	+	+	+	+	+	+	+	+	−	+	Alk.	−	+	+
XXIII. B. phytophthorus (Appel)	−	+	+	+	+	0	+	0	+	0	+	−	+	Alk.	−	+	+
XXIV. Ps. campestris (388)	−	+	÷	−	−	−	−	−	−	−	+	−	+	Alk.	−	−	−
XXV. Ps. lathyri (Clover)	−	+	÷	−	+	−	−	−	+	−	−	−	+	Alk.	−	+	−

÷ Very scanty growth under anaerobic condition.

0 Gas bubble appears after 4 days and disappears after 10 days.

Reaction in milk	Indole on peptone water	Size in μ				Spores size in μ		Motility	Flagella	Gram	Capsule	Lentil broth		Thermal death pt. of bacteria	Thermal death pt. of spores	
		Average	Biggest	Smallest	Breadth	Length	Breadth					Ring	Pellicle			
Acid	+	1·5	2·3	0·8	0·6			+	1–8 peritrichous	−	−	−	−	56° C.		I.
Acid	+	1·5	3·0	0·8	0·8			+	3–8 peritrichous	−	−	+	+	56° C.		II.
Acid	+	1·0	1·5	0·8	0·8			−	—	−	−	−	−	56° C.		III.
Acid feebly	−	1·5	1·9	1·0	1·0			+	4–6–8 peritrichous	−	−	−	−	54° C.		IV.
Neutral	−	3·0	4·5	1·5	0·7			+	2–4–6 peritrichous	−	−	−	−	50° C.		V.
Alk.	−	2·5	3·8	1·5	0·5			+	1 polar	−	−	+	+	54° C.		VI.
Alk.	−	2·3	4·5	1·5	0·8			+	3–6 bundles at 1 end	−	−	+	+	50° C.		VII.
Alk.	+	2·5	3·8	1·5	0·4			+	1 polar	−	+	+	+	54° C.		VIII.
Acid feebly	+	2·6	6·0	1·5	0·5			+	Many peritrichous	−	−	−	−	52° C.		IX.
Acid feebly	+	1·7	3·0	0·8	0·8			+	Many peritrichous	−	−	−	−	54° C.		X.
Alk.	+	3·4	6·0	1·5	0·8			+	Many peritrichous	−	−	−	−	54° C.		XI.
Alk.	−	1·5			1·1			−	—	+	−	−	−	66° C.		XII.
Alk.	−	1·6			1·1			−	—	+	−	−	−	66° C.		XIII.
Alk.	−	1·1			0·8			−	—	+	−	−	−	66° C.		XIV
Alk. feebly	−	0·9			0·9			−	—	+	−	−	−	58° C.		XV.
Alk.	+	6·0	10·5	3·0	1·5	1·8	0·8	+	2–8 peritrichous	+	+	+	+	68° C.	95° C.	XVI.
Alk. feebly	+	4·5	6·0	2·3	1·1	1·8	0·9	+	8 or more peritrichous	+	+	−	+	60° C.	90° C.	XVII.
Alk. feebly	−	3·4	5·3	2·3	0·8	1·6	0·8	+	Many peritrichous	+	−	−	+		115° C.	XVIII.
Alk. feebly	+	5·3	13·5	3·0	1·9	1·5	0·8	+	Many peritrichous	+	−	−	−	74° C	105° C.	XIX.
Alk.	−	3·0	4·5	2·3	1·0	1·2	1·0	+	Many peritrichous	+	−	+	+	72° C.	110° C.	XX.
Acid	−	1·5	2·3	1·1	0·8			+	3–5 peritrichous	−	−	−	+	52° C		XXI.
Acid	−	2·1	3·0	1·5	0·6			+	5–10 peritrichous	−	−	−	−	50° C.		XXII.
Acid	−	1·2	1·5	0·9	0·6			+	5–10 peritrichous	−	−	−	−	50° C		XXIII.
Alk.	−	1·5	2·0	1·1	0·6			+	1 polar	−	−	−	−	50° C.		XXIV.
Acid	Trace	1·1	1·5	1·0	0·8			+	1–3–6 polar	−	+	−	+	50° C.		XXV.

Diastase reaction. Strong on 15th day.

Peptone water. Turbid in 24 hours, yellow deposit on 3rd day.

Indol reaction. Negative.

Nitrate medium. Gas absent. Turbid in 24 hours. Copious yellow deposit on 4th day. Nitrate reduced to nitrite on 10th day.

Uschinsky solution. Gas absent. Alkaline in 48 hours. Slight deposit on 4th day. Litmus bleached on 10th day.

Sugar media:

Glucose. Alkaline in 24 hours; gas absent; ring present on 10th day.

Lactose. Alkaline in 24 hours; gas absent; ring present on 10th day.

Saccharose. Alkaline in 24 hours; gas absent; ring present on 10th day.

Thermal death point. 48°–50° C.

PSEUDOMONAS LATHYRI (389).

Short fat oval rods, size 1·0–1·5 μ × 0·7–0·9 μ, occurring singly, very rarely in pairs. Actively motile by means of a bundle of 3–6 long wavy flagella at one end, when the organism is united in twos a bundle of 1–3 flagella at each end. Gram negative, capsulated. It forms from light to deep yellow pigment. Aerobic, very scanty growth under anaerobic condition. No spores observed.

Gelatine plate. Round, small, smooth shiny yellow colonies with entire margin. No liquefaction is observed.

Gelatine stab. Yellow filiform growth on 3rd day. No liquefaction observed.

Gelatine streak. Yellow scanty growth on 3rd day. No liquefaction is observed.

Agar plate. Yellow shiny raised dots in 36 hours, 1–2 mm. in diameter on 3rd day. Colonies slightly irregular in outline on 6th day, with granular appearance at the centre.

Agar streak. Yellow, moist, shiny growth in 24 hours, margin crenate. Yellow granular deposit in the water of condensation in 48 hours.

Broth. Good turbidity in 24 hours, with a thin pellicle. Yellow thick deposit on 4th day.

Litmus milk. Acid on 6th day. Litmus bleached on 9th day. Coagulation positive on 16th day. No digestion of casein; it is one solid mass of pink curd on 21st day.

Potato culture. Yellow moist shiny growth in 24 hours, deep yellow on 4th day.

Diastase reaction. Strong on 15th day.

Peptone water. Good turbidity in 24 hours; yellow deposit on 4th day.

Indol reaction. Trace after 4 weeks.

Nitrate medium. Gas absent, good turbidity in 48 hours; yellow deposit on 4th day. No reduction of nitrate is observed even after 6 weeks.

Uschinsky solution. Gas absent. Good growth in 24 hours. Alkaline on 3rd day. Pellicle present on 4th day. Viscid yellow deposit on 5th day. Litmus bleached on 6th day.

Sugar media:

Glucose. Slightly acid in 48 hours. Gas absent.

Lactose. Neutral. No gas evolved. Ring present on 4th day.

Saccharose. Slightly acid in 48 hours. Gas absent.

Thermal death point. 48°–50° C.

(*Received November 11th*, 1924.)

ON THE STRUCTURE, LIFE-HISTORY, ECONOMIC IMPORTANCE AND DISTRIBUTION OF THE COCKSFOOT MOTH, *GLYPHIPTERYX FISCHERIELLA*, ZELL.

By ROSHAN LAL CHOPRA, M.A. (Punjab), Ph.D. (Wales).
Department of Zoology, University College of Wales, Aberystwyth.

(With 21 Text-figures and 4 Diagrams.)

CONTENTS.

PAGE

Introduction 359
Notes on the family Glyphipterygidae 361
Synonymy, characters and distribution of the genus *Glyphipteryx*, Hüb. . 361
Characters, description and habits of the species *Glyphipteryx fischeriella*, Zell. 363
Description of imago 363
Habits of imago 366
The egg 368
The larva—earlier stages 368
The larva—full grown 369
How to distinguish the larva in the field 377
Habits of larva 378
Cocoon 380
Pupa 381
Economic significance of the Cocksfoot Moth 383
Trial 1921 383
Trial 1922 384
Relative immunity of seeds of foreign races of cocksfoot grass . . 386
Differential injury caused to indigenous seeds 392
Loss in weight of cocksfoot seed due to damage by *G. fischeriella* . 393
Control measures 393
Distribution within and outside the British Isles 395
Summary 396
Literature 397

INTRODUCTION.

Our knowledge of Microlepidoptera in general and of the family Glyphipterygidae in particular is very meagre and incomplete. Of thirty-five genera assigned to the latter family, the early stages of more than half are yet unknown; the genus *Glyphipteryx* comprises some two hundred

and twenty species, and of these the host plants of only a dozen are definitely known.

The genus *Glyphipteryx* was first recorded in Europe by Scopoli in 1763 under the name *Phalaena* (species *Equitella* and *Thrasonella*); the total number of European species of this genus known to-day is thirteen.

The occurrence of the Cocksfoot Moth (*Glyphipteryx fischeriella*, Zell.) in Europe was first recorded by Zeller from Germany in 1839. In Britain, Stainton's record in the *Entomologists' Annual* for the year 1859 is the earliest, wherein this species, treated under the name of *G. schoenicolella*, is stated to have been discovered in Cornwall swarming amongst *Schoenus nigricans*. "On examination the seed heads were found to be eaten and an empty cocoon in the centre of many. From some of the heads collected a day or two later a few adults were reared." Since then no evidence of any damage of agricultural significance caused by this species has either been recorded in this country or on the Continent.

In 1918 for the first time the economic status of this insect was definitely established. When collecting the seed from wild cocksfoot plants in the vicinity of Aberystwyth, it was noticed that a very large proportion of the kernels had been eaten by the larvae, and that was particularly marked in the case of seed harvested from thickets. My attention was first called to the Cocksfoot Moth in 1921 and subsequent damage estimates indicate that it is of considerable interest as a pest, particularly because *Dactylis glomerata* is one of the grasses which is the subject of special investigation at the Welsh Plant Breeding Station.

The present paper embodies the results of two years' investigations into the life history of *Glyphipteryx fischeriella*, Zell., both under laboratory conditions and in natural habitats. The experiments and field observations in connection with the larval habits and the consequent economic loss were chiefly conducted at the Welsh Plant Breeding Station, Aberystwyth, where I received every facility in the collection of necessary seed material from the Director of the Station, Professor R. G. Stapledon, and several members of his staff, to whom I express my indebtedness.

I particularly wish to acknowledge my obligations to Professor R. D. Laurie, of the Department of Zoology, from whom I not only gathered helpful suggestions, but also received every assistance and advice in the preparation of this paper.

In conclusion, acknowledgment is made to the several County Agricultural Directors and other correspondents for assistance in obtaining the samples of Cocksfoot from various parts of the country on which is based the account of geographical distribution.

NOTES ON THE FAMILY GLYPHIPTERYGIDAE.

The position and composition of this family have been very much disputed. The great authority is Mr Meyrick whose work on the family Glyphipterygidae in *Genera Insectorum* is by far the most important that has yet appeared. The family comprises about thirty-five genera with some seven hundred and forty species. The moths belonging to the Glyphipterygidae are rather variable in size, form and wing coloration, the latter being often very striking. According to Meyrick the family is of great systematic importance since it gives immediate origin to Eucosmidae, Chlidanotidae and Heliodinidae, and from the first of these the three other families of Tortricina are derived. The family itself he considers to have originated in the Plutellidae, apparently from common ancestors with the genus *Protosynaema*, to which latter genus it offers a close approximation with the difference that the thickened antenna which is exhibited by *Protosynaema*, and is a characteristic of most of the Plutellid genera, is wanting in *Glyphipteryx*.

Meyrick defines the family and differentiates it from its neighbours in the following terms: "A characteristic structure of the family is the peculiar transversely compressed terminal joint of the palpi, with roughened lateral edges, but this is by no means always perceptible when well developed; however it is a very noticeable feature. The Glyphipterygidae are readily distinguished from the Eucosmidae by the absence of the basal pectination on median vein of hind-wings, and from the Plutellidae by the obsolescence of the maxillary palpi. The Heliodinidae differ by the whorls of bristles on posterior tarsi, and the Chlidanotidae by the stalking or coincidence of veins eight and nine of forewings."

SYNONYMY, CHARACTERS AND DISTRIBUTION OF THE GENUS *GLYPHIPTERYX*, HÜB.

Glyphipteryx, Hübner (4); Type *thrasonella*, Scopoli.
Lepidotarphius, Pryer (12); Type *perornatella*, Walker.
Apistomorpha, Meyrick (5); Type *argyrosema*, Meyrick.
Phryganostola, Meyrick (5); Type *drosophaes*, Meyrick.
Circica, Meyrick (6); Type *cionophora*, Meyrick.
Desmidoloma, Erschoff (3); Type *fulgens*, Erschoff.

According to Mr Meyrick (8, pp. 1-52), seven out of the thirty-five genera which the family Glyphipterygidae comprises are the development of the genus *Glyphipteryx*. As *Genera Insectorum* is expensive and not very accessible to students, I quote at length the full description of notable characters of the genus as given by Meyrick.

Head smooth; ocelli present; tongue developed, antennae 2/3 to 3/4; in ♂ shortly ciliated, basal joint moderate. Labial palpi moderate, or rarely short, curved, ascending, second joint clothed with loose scales usually forming distinct whorls, sometimes with more or less long, round, projecting tuft beneath, terminal joint as long as second or shorter, more or less transversely compressed and roughened with scales anteriorly or laterally acute. Maxillary palpi rudimentary. Posterior tibiae with oppressed scales. Forewings with 1*b* furcate, 2 from near angle, 7 and 8 sometimes stalked, 7 to termen, 11 from about middle. Hindwings 1 or less, elongate-trapezoidal or elongate-ovate or lanceolate, cilia 3/4–2; 3 and 4 connate, seldom closely approximate or short-stalked, 5 to 7 nearly parallel.

"This the basic genus of the family, shows considerable diversity in structure especially in the palpi and neuration, but the use of these characters for generic subdivision does not produce natural aggregates whilst the genus as a whole is easily recognisable. Many of the species have the characteristic habit of lightly raising and lowering the wings, with a fanning motion. They generally frequent swampy ground or open grassy places and are rather easily overlooked."

The larval stage is very much longer than the pupal and the latter as a rule is passed in a silken cocoon in which the larva rests most of the time prior to actual pupation. With regard to the food plants, Mr Meyrick says: "Food plants which are known only for six species are so far confined to orders Cyperaceae, Juncaceae, Gramineae and Crassulaceae; from the habits of the imago it is probable that most of the other species are also attached to the three first orders named. Larva feeding on seeds, or boring into shoots or flower stems."

The genus is "cosmopolitan; but by no means evenly distributed, there being more species in New Zealand than in Europe; half of the known species are from Australia and New Zealand, but probably India and South America will be found as productive when well worked; Europe, North America and Africa are certainly much more scantily supplied. In other words, the genus is more especially characteristic of the Equatorial and Southern Hemisphere except Africa. *Glyphipteryx* would seem, however, to be more probably Indian in origin, since though of cosmopolitan occurrence, it shows more diversified forms in that region than elsewhere[1]."

[1] Out of about two hundred and twenty species assigned to this genus so far, Australia supplies forty-four species, New Zealand fifty, Kermades Islands one, India and Ceylon twenty-five, Japan one, China one, Siberia four, Asia Minor two, Europe thirteen, Canaries three, Madeira one, Africa five, Seychelles two, South America fifteen, North America ten, West Indies two.

CHARACTERS, DESCRIPTION AND HABITS OF *G. FISCHERIELLA*, ZELL.

SYNONYMY.

Aechmia fischeriella, Zeller (18).
Glyphipteryx aechmiela, Duponchel (1).
Glyphipteryx desderella, Roslerstamm (13).
Glyphipteryx desideratella, Duponchel (2).
Glyphipteryx schoenicolella, Stainton (16).

DESCRIPTION OF IMAGO.

Wing expansion $7\frac{1}{2}$ to $8\frac{1}{2}$ mm.

Labial palpi three-jointed, curved, ascending and white in colour. The basal joint is shorter than the other two and is white in colour, with a black band towards its distal end; the second joint is loosely scaled and has two black bands; the terminal joint is compressed and pointed, almost as long as the second, and has one black band about the middle of its length.

Maxillary palpi small, rudimentary. Antennae fuscous, shiny, and pectinated,

Fig. 1. *Glyphipteryx fischeriella.* Adult dorsal view. ×20.

basal joints slightly thicker than the remaining portion, which is of uniform thickness. A narrow streak of whitish scales runs along the interior margin of the basal joints, thus clearly differentiating that portion from the rest. Face and head are black and smooth, sometimes dark brown and possessed of thin pubescence. Galea is thin, long and pale brown in colour. Eyes are black, large and conspicuous, surrounded by a thin brown ring, and each has a semi-circular fringe of scaliform seta along the posterior

margin, the dorsal and ventral portions of the fringe are white and the middle portion dark brown. When viewed from above, the basal boundary of head is bordered with a thick-set, light brown short fringe.

Thorax. The ventral surface is light silvery with very uneven, loosely arranged, elevated scales, which are larger in size than on any other part of the body, and have a distinctive grey sheen.

Dorsal surface of the thorax is black and bears a dark smooth ridge, thinly covered with scales or almost bare; evenly rounded and almost circular in shape along its anterior margin, but somewhat sharply pointed at the middle of the posterior margin. Legs are long and well developed, dark grey in colour. Coxa and trochanter of each pair are concealed under a thick layer of loose, silvery-grey scales. The prothoracic pair is short, thin and uniform in colour; femur only slightly flatter than the succeeding segments; the tibia and the first tarsal joint almost equal in length. The mesothoracic pair is stouter than the first, femur distinctly formed and slightly smaller than tibia. The colour is dark grey throughout, excepting at the end of the first and second tarsal joints. The metathoracic pair is much stouter than either of the two anterior pairs. The femur is broad, flat and thickly covered with bright silvery scales, particularly along the outer margin; it is much shorter than the tibia, which has in the middle a pair of stout, free and widely separated spurs. Another pair of spurs similar to the first one is situated at the junction of tibia with the tarsus, the colour of the scales at this point being silvery white as distinct from the general colour of tibia, which is dark brown. The distal end of each of the first and second tarsal segments is also marked by conspicuous white areas which are more distinctly visible from the upper than the under side. The remaining joints of the tarsus are of dark grey colour.

Forewings elongate, ovate, smooth and narrow at the apex. The basal half is coppery

Fig. 2. *G. fischeriella.* Forewing ($\times 20$); showing the arrangement of streaks and the apical ocellus on the upper side. The dotted lines denote the variations which sometimes occur in the pattern of different streaks.

brown, but the apical half is much darker in colour and near the apex tends to be almost black. Along the distal half of the costal margin there are five silvery streaks (Fig. 2). The first one is oblique and extends only halfway towards the inner margin where its lower end slightly tends to approach the second streak which is about the same length as the first or shorter. The third is generally the smallest of the series, a spot rather than a streak; the fourth is short, broader than long; the fifth is longer than the fourth and runs obliquely away from the apex, skirting the apical brilliant metallic blue-black ocellus spot resembling that which is found on each one of the larger tail feathers of a peacock.

From the inner margin a white streak generally skirts the apical spot falling short of reaching the fifth costal streak, but tending with the latter to form an arch enclosing the apical area. There is sometimes present a conspicuous streak running transversely

and tending to meet the first costal streak in the middle of the wing, as also a second transverse streak parallel with the other and tending to meet the second costal streak. Finally, a white spot may be present on the inner margin midway between the two transverse streaks of that margin. The distal third of the inner margin from 1 *b* to 6 possesses a short, thick, blackish-grey fringe. The under surface of forewings is dark brown, showing distinctly the different veins and also some traces of the spots and streaks of the upper surface. There are twelve distinct veins (Fig. 3 A); 1 *b* furcate at the base; 8–10 nearly parallel; 3–5 approximate at the base, as also 7 and 8; 3 curves markedly near the base towards 2. The entire length is 3·21 mm., and the greatest breadth about 0·75 mm.

The hindwings (Fig. 3 B) are smaller and narrower (length 2·5 mm., breadth 0·58 mm.) than the forewings, lanceolate, apex pointed, and are of a uniformly dark brown colour. The fringe of fine cilia which is of the same colour is longer (1–1·5 mm.) and thick. There are eight veins, of which 3 is short; 4–6 nearly parallel; 6 and 7

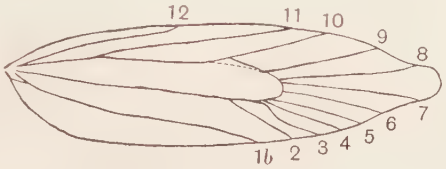


Fig. 3 A. *G. fischeriella*; showing venation of forewing.

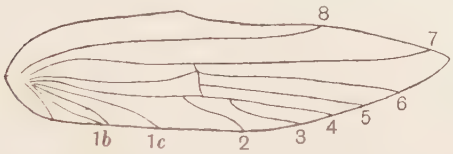


Fig. 3 B. *G. fischeriella*; showing venation of hindwing.

approximate at the base, but wider apart at their distal end; 7 almost touching the angle of the apex; 8 meets the costal margin at a point much beyond the middle, and in its distal half running almost parallel with the distal half of 7. Frenulum simple in the male, but double in the female. The lower part of each segmental division of the abdomen is marked by a white, shiny band of smooth, evenly arranged scales.

Differentiation of Sexes. The main differences concern the relative size of antennae, the abdomen, the general appearance and colour of the terminal abdominal fringe and the spines of the frenulum.

The antenna in male is thicker and slightly longer than in the female. In the male the length is about 2·1 mm., and in the female about 1·7 mm., but these figures show variation; the thickness is always a reliable distinction. The abdomen is elongated, stouter and more or less evenly rounded in the females; it is shorter, thinner and laterally compressed in the males (length, females 1·7 mm., males 1·3 mm.). When the abdomen is viewed from the ventral side, only six segments are visible in the females, but seven in the males; consequently the segments in the former sex are distinctly longer than in the latter. The last abdominal segment is fringed in both sexes, but in

the females its entire length including the fringe which is thick and straight is 0·80 mm.–0·85 mm. (Fig. 4 A). In males, the fringe is short and curved downwards at the end, parted in the centre by the harpes (Fig. 4 B) and this segment including the fringe is only 0·45 mm.–0·50 mm. long. Further, while the colour of the terminal fringe in the male is dark brown throughout, in the female the upper part is black, fuscous, with the tip clearly white.

Fig. 4 A. *G. fischeriella.* Last abdominal segment of female (upper side). ×47.
Fig. 4 B. *G. fischeriella.* Last abdominal segment of male (upper side). ×76.

The tip of the ovipositor projects beyond the fringe in both living and dead female specimens, and, similarly, the hairy harpes are easily visible between two lateral portions of the fringe in the male.

The frenulum in the male consists of a single strong spine, but in the female it is double, and forms an excellent character for distinguishing the sexes.

Habits and Manner of Dispersal of Imago.

The first adult emerged from its pupal stage under laboratory conditions, on May 21st. In the open, the moths were observed flying for the first time four days later. A few specimens, which were captured in the field on May 25th, were chiefly collected either flying round, or settled down upon nettles. On May 27th a large number were collected from gorse bushes, and a small number from nettles as well. During this period, several cocksfoot plants were carefully examined twice a day, some of which, though actually growing close to these gorse bushes, were not found to have been approached by the moths—not a single specimen being caught on the cocksfoot plants themselves. On May 31st a large number had migrated to cocksfoot plants, from which this day's specimens were chiefly collected, either sitting on the panicles, or on the leaves and stems. During the "gorse and nettle stage" of the adults, they were not noticed feeding on either of the two plants, although a close and careful examination was kept both in the open and in the laboratory.

The moth, though small and fragile, is very active, and possesses moderate power of flight. When captured in a small tube it often feigns

death, and for a few moments rests on its back motionless. On the slightest disturbance, it revives again and makes rapid movements in the cage, sliding on its back fairly long distances.

When newly emerged from the pupal stage, it is particularly restless and difficult to capture. But as the egg-laying time draws near, it becomes quieter and slower in its movements, and sometimes does not fly, even if the object on which it is settled be lightly shaken or moved.

It is diurnal in habit, the time of its greatest activity being from 2 to 5 p.m. After sunset, not a single specimen was caught on the wing, and though efforts were made several times, it was found difficult to disturb and dislodge the moth in the open, even by shaking the gorse bushes, or the cocksfoot plants. Bright, warm, windless days in June bring out the moths in large numbers, but on cold, cloudy or breezy days they remain hidden near the base of the plants. The ratio between the captures of a bright day and a dull one is 1 : 28.

Several experiments were tried, both in the field, as well as indoors, to study the behaviour of this moth towards artificial light, and it was found that it was not at all attracted by it. About two hundred moths liberated in a small room with electric light (one bulb of 30 candle-power) not only remained unattracted by it, but, on the contrary, showed a tendency to crawl away to darker spots in the room. Several which had settled down in such a place remained there overnight, and when disturbed in the morning flew towards the windows, being attracted by the light of the sun.

When at rest, this moth has the peculiar habit of raising and lowering its wings, as has been recorded for many species of *Glyphipteryx*, and swinging its body up and down along with them.

The male moths are generally the first to emerge from the pupal stage, the females appear three to four days later.

As the egg-laying time draws nearer the number of the female moths increases as compared with the males. The males have been observed to die in a day or two after copulation.

Female moths seem to have a greater power of flight than the males; are more active and more sensitive to a disturbing influence. The males, if disturbed while resting on a plant, settle down on another plant within two or three yards' distance, but the females fly from eight to ten yards before they alight to rest.

The last specimen in the open was captured on June 18th, and out of the last batch placed in captivity on June 6th, the last member died on the 11th.

The Egg.

The eggs of the Cocksfoot Moth (Fig. 5) are very small and difficult to detect without the aid of a lens. They are oval, elongate, measuring 0·38 × 0·14 mm., and are pearly white in colour when newly laid, but as they mature they acquire a strong greenish tint. The outer shell is finely sculptured all over with small darkish patches which are connected one to another by a network of thin short lines. The eggs are laid singly, and are most commonly to be found at the base of the seeds somewhere in the centre of a panicle where they are well protected; therefore when the larva hatch they find themselves on that part of the seed where the kernel—their future food—is at its thickest.

Fig. 5. *G. fischeriella.* Egg. × 130.

The female is capable of laying from twenty-five to thirty eggs, the highest number discovered on one panicle being eight, though as many as thirteen larvae have been found feeding on one and the same panicle; some of these had, however, probably migrated from other panicles growing close thereto. The egg hatches out in June in from ten to fifteen days' time.

The Larva—Earlier Stages.

The newly hatched larva is glassy white in colour, measuring about 1·2 mm. in length and is a fairly active creature. The head and the anal plate are light brown and the setae on them, as well as on the body, are long and distinct. After about twenty-four hours the ventral markings appear.

About the third day, the first moult occurs, as a rule inside the seed on which the young larva has fed, and the larva is now 1·7 mm. in length; at this stage the dorsal setae are well marked, the prothoracic shield which at hatching was faint is now distinctly developed and is 0·17 mm. and the anal plate 0·21 mm. in length. About three days later the second moult occurs, the larva being nearly pure white, very active and restless, wrinkling itself up at the lightest touch. The extreme length is 2·5 to 3 mm., and the divisional segments of the body are well marked, the head, the thoracic shield and the anal plate are all reddish-brown in colour. The ventral markings are pinkish and distinctly visible. The abdominal spiracles as well as the thoracic are clearly to be seen, the latter being

more highly chitinised on their exterior borders than the former. In short, at this stage of growth, the larva possesses certain distinguishing features which are retained till it attains full growth. The arrangement of setae, of course, differs at different periods, but in its general appearances and for the purposes of indentification it is almost the same as when full grown.

Two further moults follow with a week's interval between each, the latter of the two taking place some time after the larva has finished spinning the cocoon for its long retirement. The colour of the body tends to become distinctly greener as the larva feeds and grows.

THE LARVA—FULL GROWN.

The figures in the following table represent the typical measurements of the full-grown larvae; there is, of course, some individual variation:

Measurements of full-grown larva.

Measurements	Breadth mm.	Length mm.
Whole larva	0·85	5½–6
Head capsule (dorsal aspect)	0·47	0·45
Longitudinal ridge	—	0·11
Adfrontal suture	—	0·28
Adfrontal ridge	—	0·17
Antennae	—	0·07
Mandibles	0·14	0·14
Entire prothorax	0·86	0·72
Prothoracic plate	0·61	0·28
Ventral patches on body segments	0·08	0·11
Longest abdominal segment	1·1	0·20

General Account of Head. The full-grown larva measures 5½ to 6 mm. in length. The body is elongated and cylindrical, gradually tapering towards the hind end (Fig. 6).

The colour is of medium green or pale shade owing to the assimilation of food. The head of the larva is a smooth, chitinous, shiny, retractile structure, dark brown in colour. Viewed from above it is almost spherical in shape, nearly circular in outline, and a little broader than long (length 0·45 mm., breadth 0·47 mm.). The greatest width is a little below the middle; distance between the dorsal extremities of the hind margin is about one-third of the greatest width of the head; the incision of dorsal hind margin is one-fifth of the diameter of the head. Adfrontal suture is more or less undulating and reaches the incision of the hind margin. The adfrontal ridges of frons converge towards the middle, meeting at the point of attachment of tentorial arms, to be continued as a longitudinal ridge which is nearly one-third of the length of the entire front. Projection of dorsal margin over the ventral is nearly one-third of the breadth of head. If the head be examined from the ventral side, on each side of the labrum can be seen a dark comparatively stout and highly chitinised mandible

(Fig. 7), squarish in shape with a broader base, around articulating knob and one long and one shorter seta on the exposed surface near the outer edges.

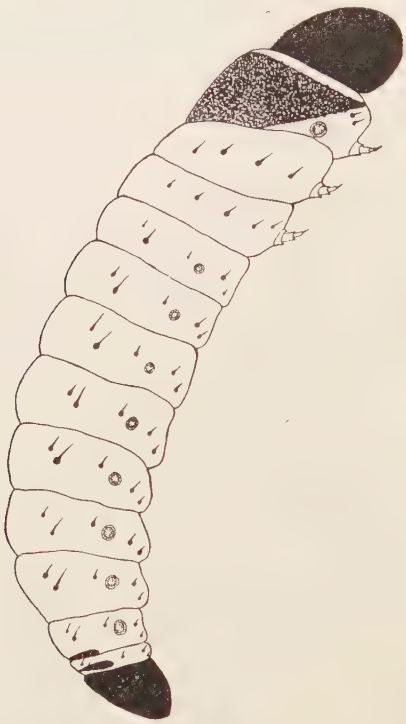


Fig. 6. *G. fischeriella.* Full-grown larva; side view. ×20.

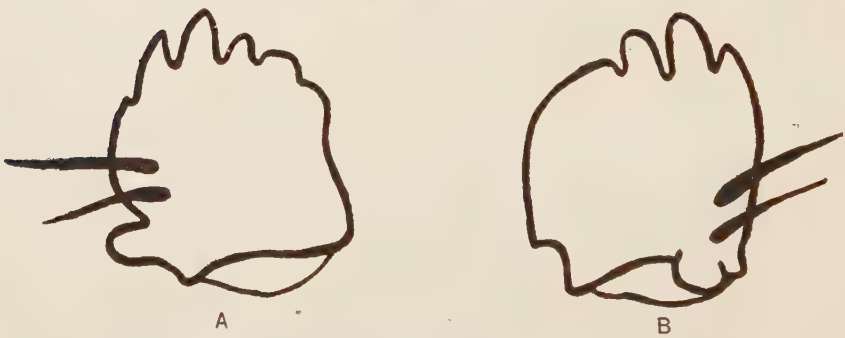


Fig. 7 A. *G. fischeriella.* Right mandible of larva from under side. ×210.
Fig. 7 B. Left mandible of larva from under side. ×210.

Each mandible is possessed of four teeth—the outer three being long, well-developed and distinct, but the fourth, represented only by a small, broad and blunt chitinous ridge which is more conspicuous in the case of right mandible than the left. In addition to these, the boundary line of the outer margin of the right mandible is broken

by a small, tooth-like process. Of the four proper teeth, the second one is sharper and larger than the others.

Epipharyngeal shield, is a small chitinised edge portion of the median incision,

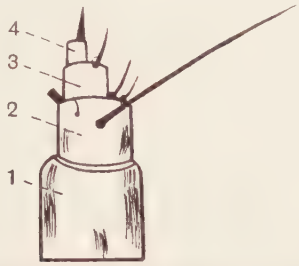


Fig. 8. Right antenna of larva from upper side. ×470.

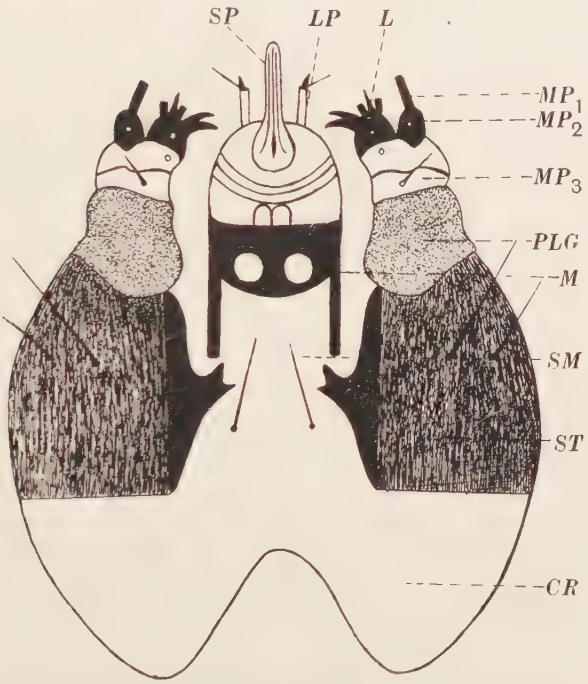


Fig. 9. Larval head from under side; showing labium and maxilla. ×150.

SP, spinneret; *LP*, labial palpus; *L*, lacinia and galea; *M*, mentum; *SM*, submentum; *CR*, cardo; *ST*, stipes; *PLG*, palpiger; MP_1, maxillary palpus, first joint; MP_2, maxillary palpus, second joint; MP_3, maxillary palpus, third joint.

and the epipharyngeal setae, of which there are three pairs altogether, are in the shape of narrow plates, and are grouped triangularly and placed near the anterior margin. The posterior projections of epipharyngeal rods are quite well developed.

Antennae (Fig. 8) very small and four-jointed. The second joint is longer and

broader than either the third or the fourth, and bears a stout seta which is longer than the entire antennae.

It also bears a pair of small setae at its apical extremity, and another still smaller sub-apically. The third and the fourth joints also possess apically a small seta each.

Looking at the head ventrally, one can easily distinguish the maxilla situated anteriorly. The maxillary palps (Fig. 9) are three-jointed, galea and lacinia well developed, the former consisting of two dagger-like processes.

Palpiger is thick, slightly bulging towards the inner side.

Stipes is a strong chitinised portion, regularly curved along the outer margin, but bearing along the inner margin two tooth-like projections.

Labial palps are situated one on each side of the spinneret, are two-jointed, of which the first one is about five times the length of the second which bears a seta at its distal margin of about double its own length. Between these palps is the spinneret which is very minute and consists of three thin, thread-like processes, the middle one being similar to the blade of a dagger, only pointed at both ends.

The labrum (Fig. 10) has a rather deep and very evenly rounded median incision

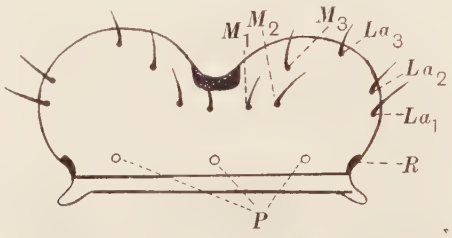


Fig. 10. Labrum of larva. ×300.

La_1, La_2, La_3, lateral labral setae; M_1, M_2, M_3, median labral setae; P, labral punctures; R, epipharyngeal rod.

and possesses six setae on each side, following the lateral and anterior margins more or less closely; three punctures follow the posterior margin, two of them lateral and one median. Two of the lateral setae (La_1 and La_2) are situated close to each other near the lateral margin; La_3 is close to the anterior margin. The median seta, M_3, is less close to the margin, and together with other two median setae (M_1 and M_2) forms the half of a semicircle whose convexity is directed posteriorly.

Seta arrangement of Head Capsule (dorsal view). In frontal region are a row of four epistomal setae, the middle two of which are further from each other than each is from the outer epistomal seta of its own side (Fig. 11). Behind and to the inner side of these are another pair of setae.

The adfrontal region of each side bears two setae, one of these is about the middle of the area, and almost a third of the distance along the adfrontal ridge from the fork; the other is a little way behind the fork.

On the epicranial region of each side are (following Heinrich's terminology) an anterior group comprising three setae (A_1, A_2, A_3) and one puncture (Aa); a posterior group comprising three setae (P_1, P_2, P_3) and two punctures (Pa, Pb); a lateral group comprising one seta (L_1) and a puncture (La); and an ultra-posterior group of three punctures X, Y, Z.

A_1, A_2, A_3 and L_1 form a series running back in a line laterally over the anterior half of the epicranium and equidistant from each other. P_1, P_2 and Pa are on a level with the anterior adfrontal setae and together with the corresponding setae and puncture of the opposite side form a transverse series, Pa being above and to the inner side of the space between A_3 and L_1. La is above, to the inner side of and behind L_1. The remaining punctures and setae, namely Pb, P_3, X, Y and Z form a series running backwards and outwards over the posterior half of the epicranium. A line joining these would present a sigmoid curve; a line joining P_3 and X would run parallel to a line joining Pb, Y and Z; the space between P_3 and X is greater than between any other two successive members of the series. Pb is behind the space between P_1 and P_2.

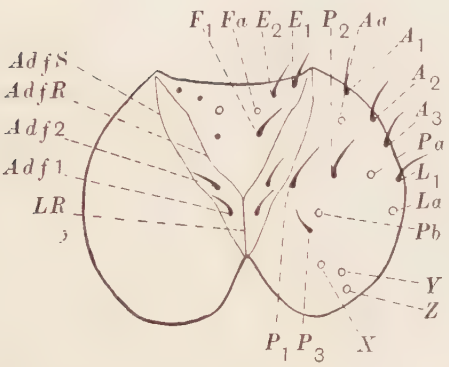


Fig. 11.

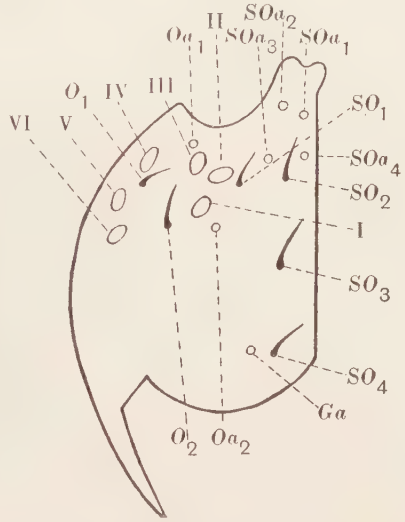


Fig. 12.

Fig. 11. Head capsule of larva from dorsal view and its seta arrangement. × 80.

AdfR, adfrontal ridge; *AdfS*, adfrontal suture; *LR*, longitudinal ridge; A_1, A_2, A_3, anterior setae; *Aa*, anterior puncture; P_1, P_2, P_3, posterior setae; *Pa*, *Pb*, posterior punctures; L_1, lateral seta; *La*, lateral puncture; *X*, *Y*, *Z*, ultra-posterior punctures; Adf_1, Adf_2, adfrontal setae; E_1, E_2, epistomal setae; F_1, frontal seta; *Fa*, frontal puncture.

Fig. 12. Seta arrangement of epicranium (ventral view). × 168.

I, II, III, IV, V, VI, ocelli; O_1, O_2, ocellar setae; Oa_1, Oa_2, ocellar punctures; SO_1, SO_2, SO_3, SO_4, sub-ocellar setae; SOa_1, SOa_2, SOa_3, SOa_4, sub-ocellar punctures; *Ga*, genal puncture.

Seta arrangement of Epicranium (ventral view). There are six ocelli arranged in an oval (Fig. 12). Ocellus No. II is very close to ocellus III, being so placed as to draw the oval to a point antero-ventrally; there is a considerable space between ocelli I and VI.

Inner (ventral) to the level of ocelli are four sub-ocellar punctures (SOa_1, SOa_2, SOa_3, SOa_4), and four sub-ocellar setae (SO_1, SO_2, SO_3, SO_4). The punctures form a more or less quadrilateral arrangement anteriorly. Of the setae, SO_1 is inner (ventral)

to the space between ocelli I and II, the other three form an antero-posterior line, the anterior one (SO_2) being at the same horizontal level as and ventral to SO_1. SO_3 is equidistant between SO_2 and SO_4. SO_4 might be more properly termed a genal seta and a little to the outer side is the genal puncture. There are two ocellar seta (O_1 and O_2) and two ocellar punctures (Oa_1 and Oa_2). Seta O_1 is immediately to the inner side of the space between ocelli IV and V; seta O_2 is between ocelli VI and I. Of the ocellar punctures, Oa_1 is very close to the antero-dorsal margin of ocellus III; Oa_2 is posterior to and somewhat to the inner (ventral) side of ocellus I. Well behind ocelli and on the lateral margin is the lateral seta.

General Account of Thorax. Viewed from above, the prothorax is the biggest division of the body of the larva. The posterior margin is semicircular, the anterior margin a straight conspicuous rim of glistening dirty white colour beneath which about one-third of the posterior portion of the head capsule may be withdrawn. The prothorax bears

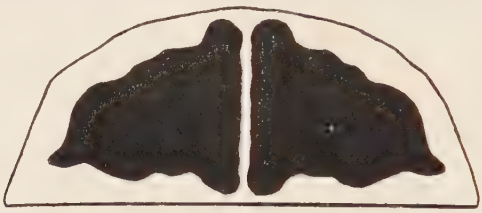


Fig. 13. Dorsal prothoracic shield of the larva. × 80.

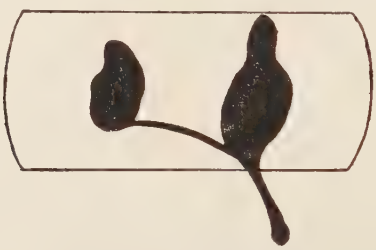


Fig. 14. Fifth abdominal segment of larva, showing two black bodies on the dorsal surface. × 60.

a shiny dark brown shield (Fig. 13), extending over almost the entire dorsal length of the segment, and divided in the centre by a narrow, uneven suture, running antero-posteriorly across it.

The meso- and meta-thorax are of uniform size and colour. Each of the three thoracic segments bears ventrally a pair of strong and well-developed legs, each of which terminates in a short, slightly curved hook, enclosed between two processes.

The prothoracic pair of spiracles is larger than the abdominal pairs and is also situated comparatively more ventro-laterally. Slightly to the inner side of each spiracle and almost touching it is placed a small light brown chitinous patch.

General Account of Abdomen. Abdomen, which consists of ten segments, when viewed from above does not show any peculiarities in the case of the first six segments, all of which are somewhat cylindrical in shape and almost equal in size. In the majority of larvae about two months prior to pupation there appear two oblong, irregular black subcutaneous bodies (Fig. 14), occupying the posterior half of the fifth and the anterior

half of the sixth abdominal segments. As a rule, these black patches can distinctly be seen till pupation takes place, but in certain cases they disappear a week or two before the larva changes into the pupa.

The seventh segment is the first to narrow down a little, and each succeeding segment is narrower than the one previous to it, the last bearing the anal plate is the narrowest. The middle half of the posterior extremity of the eighth abdominal segment is slightly dark and chitinised. The ninth abdominal segment bears a single small, oblong, light brown plate (Fig. 15 A), extending transversally across the segment. The lateral edges of this plate are narrowed and rather pointed.

The central portion is also somewhat narrowed. The tenth and last segment consists of a single, thick and heavily chitinised plate, rather deep brown in colour; the anterior margin straight and almost double the width of the posterior portion which is slightly rounded (Fig. 15 B).

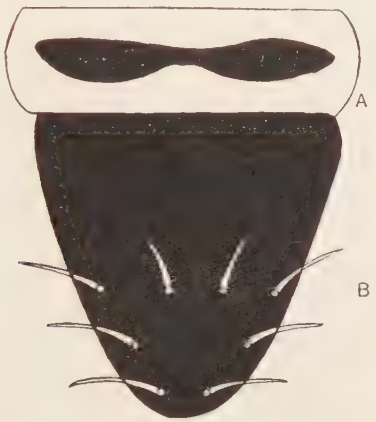


Fig. 15 A. Dorsal view of plate on the ninth abdominal segment of larva. ×150.
Fig. 15 B. Dorsal view of anal plate of 7th larva. ×140.

There are eight pairs of spiracles circular in shape and composed of a double ring, the inner one being darker and more distinct than the outer. The pair on the eighth abdominal segment is slightly larger than those on the first seven abdominal segments.

Abdominal feet, of which there are the five usual pairs, are small, rounded, elevated structures, supplied each with a pair of minute hooks only.

Seta arrangement on dorsal surface of Thorax and Abdomen. In a dorsal view, there are seen six longitudinal rows of setae roughly equidistant from each other (Fig. 16). They comprise a median pair of rows, clearly dorsal in position: a dorso-lateral pair to the outside of these and to the inner side (dorsal) of the spiracles; and a ventro-lateral pair near the lateral tergal margin, seen clearly when the larva is viewed from the side.

On detailed examination it will be found that with regard to the seta arrangement, the first thoracic and last abdominal segments are characteristic and the remainder fall into two groups, one comprising the second and the third thoracic and the other the first nine abdominal segments.

In the first thoracic segment, each half of the prothoracic plate bears nine setae, of which six form an irregular transverse row close to the anterior margin; and the remaining three form a series running obliquely backward and outwards from behind the innermost member of the anterior series. To the outer side of the plate there are two small setae some distance in front of the spiracle.

The typical abdominal segment has the following arrangement of setae. Two pairs, of which the posterior is the larger, represent the median rows; a single seta on each side represents the dorso-lateral element and two setae on each side represent the lateral, sub-marginal (ventro-lateral) row. All these setae, excepting the two posterior median and the two posterior lateral ones, form a transverse series in the anterior portion of the segment.

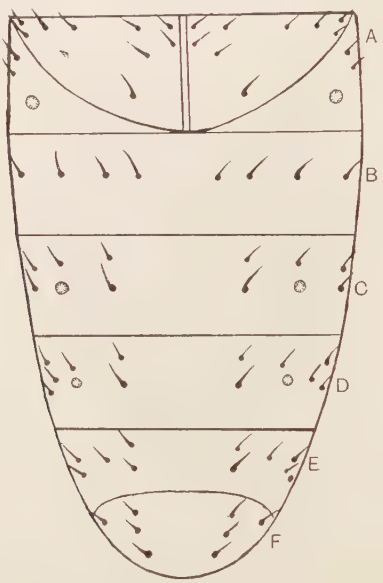


Fig. 16. Seta arrangement on dorsal surface of thorax and abdomen of larva.
A, first thoracic segments; *B*, second thoracic segment; *C*, *D*, *E*, *F*, 7th, 8th, 9th, and 10th abdominal segments respectively.

The second and third thoracic differ from those of the abdominal group by the absence of the posterior median pair, and the presence of a smaller pair to the inside of and close to the anterior median pair.

The tenth abdominal segment bears three pairs of setae representing the median rows, and there is in addition on each side a laterally placed seta.

Ventral patches on Thorax and Abdomen. The ventral surface of each of the thoracic, as well as of the first seven abdominal segments of the larva possesses an almost circular dotted patch (Fig. 17) of pinkish tinge, with the margin and antero-posterior diameter darker and more distinctly marked than the rest. The abdominal patches are situated on the anterior extremity of each body segment, excepting that the seventh segment has two patches; one in the usual position and the other posteriorly,

appearing as one belonging to the succeeding (eighth) segment. The thoracic patches are in each case located almost in the centre of the segments, and at the basal junction of the thoracic feet. The prothoracic patch is different from all the rest in being made up of two distinct and full-sized patches, almost touching each other, and extending over the full length of the segment. The anterior one of the two is oval in shape like the rest, but the posterior one is hemispherical in shape with the rounded margin posterior. On viewing the patches as a series, it is observed that they are equidistant from each other, except that the last two are closer together and also the meta-thoracic and the first abdominal (Fig. 17).

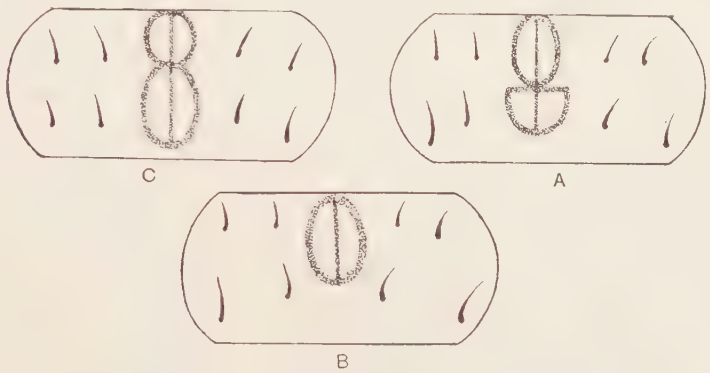


Fig. 17. Ventral patches. A. Prothoracic patch. B. Patch of 2nd and 3rd thoracic and first six abdominal segments. C. Patch of 7th abdominal segment.

HOW TO DISTINGUISH THE LARVAE of *G. FISCHERIELLA*, ZELL. IN THE FIELD.

Glyphipteryx fischeriella is one of the smallest Lepidoptera known to cause somewhat serious damage to an important agricultural crop in the field. For a definite determination of its larva, which is the sole agent of damage, the aid of the microscope is essential; but the combination of the following characters, which can be discerned by the aid of a common pocket lens, will enable the practical worker to arrive at a reasonably certain preliminary determination.

If a small green, or greenish-white larva (about 6 mm.), body cylindrical in shape and somewhat abruptly tapering towards the hind end, be found on or near a cocksfoot plant, the seeds of which on examination are found to have small circular holes in them and are hollow inside, the larva in all probability is that of *G. fischericlla*.

On closer examination the following characters will be noticed:

(1) The head is blackish-brown and retractile. The prothorax, which is the largest segment of the body, has on its dorsal surface a dark brown shield made up of two portions which are distinctly separated from each other by a light brown, perpen-

dicular, uneven suture line. The thoracic pair of spiracles is marked by its highly chitinised edges, and is much larger and more conspicuous than the abdominal ones.

(2) The last abdominal segment consists of a completely chitinised dark brown plate, somewhat triangular in shape and containing eight strong, tubercular setae, as shown in Fig. 15 B.

(3) On the ventral surface, the larva possesses twelve distinct, oval, pinkish patches, arranged in a longitudinal series, each being vertically divided into two equal halves by a faint dotted line. Of these, two are situated on the prothoracic segment, one each on the other two thoracic segments, and one each on the first six abdominal segments, and two on the seventh segment.

(4) The ninth segment, altogether concealed in a ventral view of the larva, but viewed dorsally is possessed of a dark narrow shield (Fig. 15 A). The central half of the posterior extremity of the eighth abdominal segment is also slightly dark and somewhat thickened.

(5) On the dorsal surface of the larva, there are six distinctly visible longitudinal parallel rows of setae (Fig. 16).

(6) The prolegs are represented only by small, round, slightly elevated stumps.

As a rule, the larva of the Cocksfoot Moth is to be found in the field from the middle of June to the third week in July.

Habits of Larva.

The young larva on hatching makes its first meal off the empty shell, and six to ten hours later starts boring into the same seed on which it was hatched. Sometimes it wanders away from the actual spot where the egg was laid, but in either case it remains close to the bottom of the seeds. As a general rule, the entrance holes (Fig. 18 A) cut by the larvae are to be found more towards the bottom of the infected seed, where the kernel is at its thickest, than towards the top, with the result that unless seeds are separated and carefully examined, it is well-nigh impossible to detect an infected panicle from a healthy one, in the field.

After having gained an entrance into the seed the larva closes the hole by means of a rough, irregularly spun web. It proceeds to consume its food, which it does at the rate of one to two seeds in from three to five days. After the first seed is completely hollowed, the larva comes out of the empty husk, and either attacks a new one further away or bores into an adjacent seed (Fig. 18 B). There is always only one hole in each attacked seed.

When full fed it often crawls out of the seed and having found a suitable place for pupation spins a cocoon in which it rests during the winter months.

From several experiments which I tried in order to test the sight and sense of direction of these larvae, it appears that their sight is poor.

Even large objects placed close to them failed to attract, while much smaller and distantly placed objects which chanced to be in their way were sometimes selected as a resting place. When travelling in the open, they do not speedily change the direction of their march; even if an obstacle be placed in their way they try to surmount it. Even their food plants placed in their way did not attract them any more than other objects of the same size. Their sense of hearing seems equally poor; but on the other hand, they respond to the slightest touch, and at once curl up and stop all movement. If placed on an object high above the ground, they manage to alight therefrom by hanging by a thread. Several have been

Fig. 18 A. Cocksfoot seed infested with the larva of *G. fischeriella*, and showing the entrance hole cut by the larva.

Fig. 18 B. Few adjoining seeds each tunnelled by the larva from inside the other. ×10.

observed to reach the ground in this way from a height of about seven feet in less than ten minutes. It is very rarely that a larva is seen travelling downwards on foot. They are very sensitive to a rise in temperature and if at the full-grown stage they be subjected to a slightly higher temperature than that of the atmosphere they commence to construct their cocoons earlier and to complete them a little sooner than those which are left under natural conditions, or those which have been kept immersed in iced water in an airtight tube.

After the first moult, which takes place as a rule within the seed, the larva is very active and restless, and if slightly disturbed comes out of its feeding chamber and begins to wander about in search of a resting place.

Cocoon.

Length $5\frac{1}{2}$ to $6\frac{1}{2}$ mm.

Nearly 28 per cent. of about six hundred larvae that were put in a breeding-jar, together with their food plant on July 15th, were found on re-examination on the 17th to have constructed cocoons inside the hollow husk of the seeds which had previously served as their food. The majority of them spun cocoons either in the hollow cavity of a spikelet, or in the space between two seeds (Fig. 19).

About 6 per cent. of the larvae climbed up to the hollow space under the top rim of the breeding-jar and there they chose their resting place. Of all the cocoons, those of the last kind are most regular in shape and structure.

Fig. 19.

Fig. 20.

Fig. 19. Showing a cocoon constructed in the cavity between two growing seeds on a panicle. ×10.

Fig. 20. Cocoon of the larva of *G. fischeriella*. ×14.

On July 12th, for the first time, certain larvae were observed to have started spinning. Prior to this they were very active and somewhat restless when disturbed.

The cocoons (Fig. 20) are irregularly elongate-oval, the usual length being 6 mm., and the breadth varying according to the situation.

In construction, the cocoon has a thin, translucent wall, composed of an intricate network of fine silken threads. When freshly constructed, it is pure white in colour, but in the course of a day or two it becomes dirty white mixed with light grey, a colour which it retains to the last. As a rule, the outer surface is slightly rougher and looser, but the inner surface is smoother and more compact in texture; in most cases the green larva can be indistinctly seen inside it, even with the naked eye. Inside the cocoon, the larva is always contracted and does not occupy more than approximately three-fifths of the space actually required for its full length.

Actual spinning operations last from about eighteen to twenty-one hours. The first batch of larvae which commenced spinning at about 4 o'clock in the afternoon of July 12th, were found to have completely covered themselves by the following morning.

If the larva is taken out of the cocoon, even a day or two after the construction is completed, it does not attempt to spin a new one. The last moult occurs some time after the formation of the cocoon and the cast off skin is utilised by the larva for consolidating the inner layer of the cocoon; this fact becomes quite evident by comparing the structure of a freshly spun cocoon with the one after the last ecdysis.

Generally speaking, the larva, prior to actual pupation, bites its way out of the cocoon and either pupates outside, or the pupa is to be seen protruding from the cocoon, but in a few cases when pupation takes place inside the cocoon the larva also bites a hole to facilitate the emergence of the adult.

Pupa.

Length 3·25 to 3·50 mm. Greatest width about 0·88 mm. Breadth across region of base of genital openings 0·28 mm.

The general colour is smoky brown in some, in others a pale tinge which at the end of the first week of the pupal stage assumes darker shades. The intersegmental membranes are reddish, a tinge which they retain till the time of emerging.

It is rather plump and robust in shape (Fig. 21 A), of more or less uniform width from the metathoracic segment to the fifth abdominal whence it narrows down rapidly towards the posterior end which is rounded. The last four segments are movable. The entire surface of the body is smooth, and the abdominal divisions are distinct. The head is ventral.

Pupal eyes, clypeus and labrum are distinctly indicated, mandibles less so. Front part of clypeus suture is distinctly and abruptly curved upwards near median line. The antenna extends to the ventral edge of the third abdominal segment; metathoracic legs almost touch the anterior boundary line of the eighth segment and reach slightly beyond the extreme tip of the wings, which terminate with the anterior margin of the seventh segment. The wings are finely sculptured. Genital opening, which is comparatively large and slit-like, is surrounded by one strong and hooked

seta, and two smaller setae on each side. Cremaster is not developed, and the anal opening on the tip of the abdomen ventrally is not armed with any seta (Fig. 21 A). There is no evidence of larval prolegs. When mature, the pupa becomes darker and reddish-brown and the eyes of the imago are discernible from under the genae of the pupal skin.

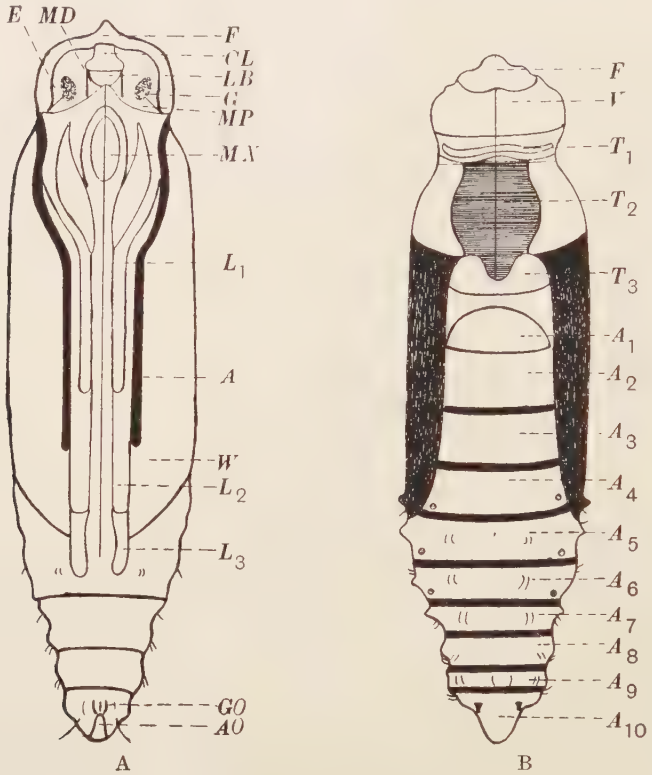


Fig. 21 A. *G. fischeriella.* Pupa from front. ×30.

LB, labrum; *F*, front; *CL*, clypeus; *E*, eyes; *MP*, maxillary palps; *G*, gena; *MD*, mandibles; *MX*, maxilla; L_1, L_2, L_3, three thoracic legs; *A*, antenna; *W*, forewings; *GO*, genital opening; *AO*, anal opening.

Fig. 21 B. Pupa from back. ×30.

F, front; *V*, vertex; T_1, T_2, T_3, three thoracic segments; A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8, A_9, A_{10}, ten abdominal segments.

The anterior, as well as the posterior, boundary of vertex is elevated dorsally (Fig. 21 B). The mesothorax bears a strong central longitudinal ridge, which extends posteriorly on to the metathorax. The first thoracic segment is distinctly marked off from the second by a dark depressed line. The spiracles are small, slightly elevated, dark brown in colour and oval in shape. The last six abdominal segments bear four pairs each of small light brown setae, situated more or less in parallel rows; the last but one possesses an additional pair.

The newly formed pupa s very active and if slightly disturbed twirls the movable segments of the abdomen with circular motion. Duration of pupal stage is from three to four weeks and sometimes longer.

Table of measurements of average pupa, whose greatest length was 3·43 mm.

Measurements from front end to	Length mm.
End of antennae	2·28
End of first pair of legs	1·71
End of mesothoracic legs	2·93
End of mesothorax	0·92
Posterior end of maxilla	0·71
Tip of mesothoracic wings	2·74
Posterior margin of first abdominal segment	1·42
Base of genital opening	3·28

ECONOMIC SIGNIFICANCE OF *GLYPHIPTERYX FISCHERIELLA.*

One of the difficulties which has, more or less, discouraged the cultivation of cocksfoot grass in this country lies in the fact that the best seed employed here at present comes chiefly from Denmark, America and New Zealand, and it has been found increasingly difficult to obtain the supply. With a view to obviating the difficulty of importing the seed from abroad, the question of growing the species in this country has lately been receiving special attention. At the Welsh Plant Breeding Station, Aberystwyth, cocksfoot is the subject of a critical study and investigation. It was there that I first noticed the occurrence of the Cocksfoot Moth in June 1921, when several specimens were caught flying round the flower-stalks of cocksfoot plants. At a later date, the seeds harvested from these plants were, on examination, found to be severely damaged by the larvae of the moth. In the case of each attacked seed the kernel inside was completely consumed and only the hollow husk left, the damage being easily noticeable from the small circular holes cut by the larvae.

TRIAL, 1921.

In the 1921 trial the material examined for the detailed estimate of larval damage mainly consisted of four different "nationalities" of the cocksfoot seed grown at the Welsh Plant Breeding Station that year, viz. American, Danish, French, and Indigenous. Each of these "nationalities" was grown in separate adjacent plots, or in parallel rows. The adult moths had an almost equal chance of reaching the plants of any "nationality" and in fact almost all the plots were noticed to be frequented by them in fairly large numbers.

Besides the seeds of cultivated indigenous plants which were growing on plots in the garden of the Plant Breeding Station, a large amount of seed for examination was collected at random from wild cocksfoot plants growing in hedges on the roadside and in waste places in the vicinity of Aberystwyth. A few specimens also were obtained from about a dozen different localities in the County of Cardigan within ten miles of Aberystwyth, among which were Borth, 6 miles north of Aberystwyth; Capel Bangor, $4\frac{1}{2}$ east; Ponterwyd $10\frac{1}{2}$ east; Minffordd, $2\frac{1}{4}$ south-east; Llanychaiarn $2\frac{1}{4}$ south; Llangorwen, 2 north; Broncastellan, 4 north-east; Pencraig, $5\frac{3}{4}$ south-east; Llanilar, 5 south-east; Rhostie $7\frac{1}{2}$ south; Llancynfelin, $7\frac{1}{2}$ north, Hirnani, $10\frac{1}{2}$ north-east; Llangwyryfon, $8\frac{1}{2}$ south; Capel Seion, 3 east; Aberystwyth town. In the month of October a careful examination of all the material harvested that year was made, and the following results were obtained:

1921 *Crop.*

Nationality of seed	Number of samples examined	Approximate number of seeds in each sample	Average percentage of damage	Maximum percentage of damage	Date of harvest
American	14	39,490	0·71	2·1	July 5th
French	5	11,467	1·41	4·71	„
Danish	17	25,520	1·29	4·2	„
Indigenous (cultivated)	12	52,209	18·76	63·8	„
Indigenous (growing wild in natural habitats)	21	752	24·21	41·3	Between July 9th and August 3rd

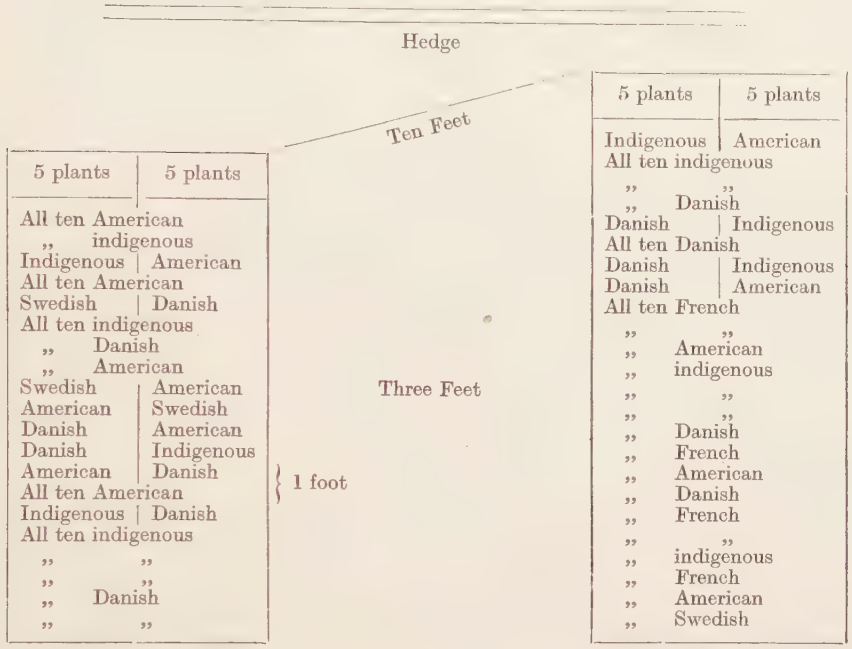
Trial 1922.

The 1922 trial for determining the economic status of the Cocksfoot Moth as an agricultural pest was arranged on a more elaborate scale than that of the previous year. Through the kindness of Prof. Stapledon, Director of the Plant Breeding Station, I was enabled to secure a plot of land which contained about five hundred plants of five different "nationalities" of cocksfoot. It consisted of two parallel, rectangular plots, each measuring twenty-five feet by six feet, and about three feet apart. Each of the plots contained twenty to twenty-four rows of ten plants each, which were about one foot apart. Some of the rows had all the ten plants of one and the same "nationality," while others were composed of two different "nationalities" of five plants each. With a view to securing as equal a distribution of the pest over the area as possible, the plants were grouped in such a way that by various combinations those

of all the "nationalities" were in a close proximity with one another in one or other of these rows. The "nationalities" experimented with were the same as in the previous year, with the addition of Swedish.

The following plan of the experimental plot will illustrate the order of arrangement:

Plan of Experimental Plot Sown with different Nationalities of Cocksfoot.



At the various stages of the growth of these plants, both before and after the period of flowering and at the period of maximum flowering, a few hundred moths were liberated in both of these plots at different times during the day. At the distance of about ten feet from these plots was a hedge actually swarming with these moths, which were noticed to frequent the plants in fairly large numbers. The seeds were harvested at different dates after ripening. The indigenous between July 2nd and 8th, the French between July 4th and 8th, the Danish between July 3rd and 11th. The seeds of wild indigenous cocksfoot (natural habitats) were gathered at random between July 4th and 19th from fifteen different places in the county, all within twenty miles of Aberystwyth, but different from the localities of the previous year, and included the

following: Crosswood, 9 miles south-east of Aberystwyth; Devil's Bridge, 12 miles south-east; Tregaron, 17 miles south-east; Machynlleth, 18 miles north-east; Llangeithio, 14 miles south-east; Hafod Rhyd, 16 miles south-east; Clarach, 2½ miles north; Llanarth, 19 miles south; Pwll Peiran, 17½ miles east; Blaenpennal, 13 miles south-east; Melindwr Pau, 11½ miles east; Henbant, 12 miles south-east; Bryn Hope, 15 miles south-east; and Trepeddian, 13–14 miles north.

The whole material composed of the various five "nationalities" was carefully examined immediately after harvesting. The results calculated are set out in the following table:

1922 *Crop.*

Nationality of seed	Number of samples examined	Approximate number of seeds in each sample	Average percentage of damage	Maximum percentage of damage recorded	Date of harvest
American	15	9,490	1·47	5·6	From July 5th–12th
French	15	4,774	2·33	5·2	„ July 5th–12th
Danish	15	9,862	2·31	6·2	„ July 4th–11th
Swedish	24	1,053	9·74	20·9	„ July 5th–8th
Indigenous (cultivated)	20	12,547	15·62	21·03	„ July 5th–8th
Indigenous (growing wild in natural habitats)	20	3,538	33·16	62·79	„ July 3rd–12th

The results of the various observations which are contained in the foregoing tables are set out in Diagrams I, II, III and IV.

On comparing these curves, several striking points will be noted. Firstly, that the average amount and range of damage shown by the seeds of the different groups is very similar in the two years. Secondly, that the seeds of indigenous cocksfoot suffered far more severely than those of the foreign "nationalities." Thirdly, that among the indigenous plants, the wild-growing ones suffered more than the cultivated.

Relative Immunity of Foreign Seeds.

With regard to the relative immunity of the foreign seeds as compared with the indigenous, four hypotheses may be discussed by way of attempted explanation: (*a*) that the egg-laying period of the moth is correlated with the flowering period of the plant; (*b*) that the egg-laying is correlated with the colour of the panicles of the plant; (*c*) that the eggs are laid upon the panicles indiscriminately, and that upon hatching the larvae migrate to palatable seeds of suitable consistency; (*d*) that

Diagram I. Showing the percentages of damage due to *G. fischeriella* upon series of samples of seeds of different "nationalities" of cocksfoot harvested in 1921.

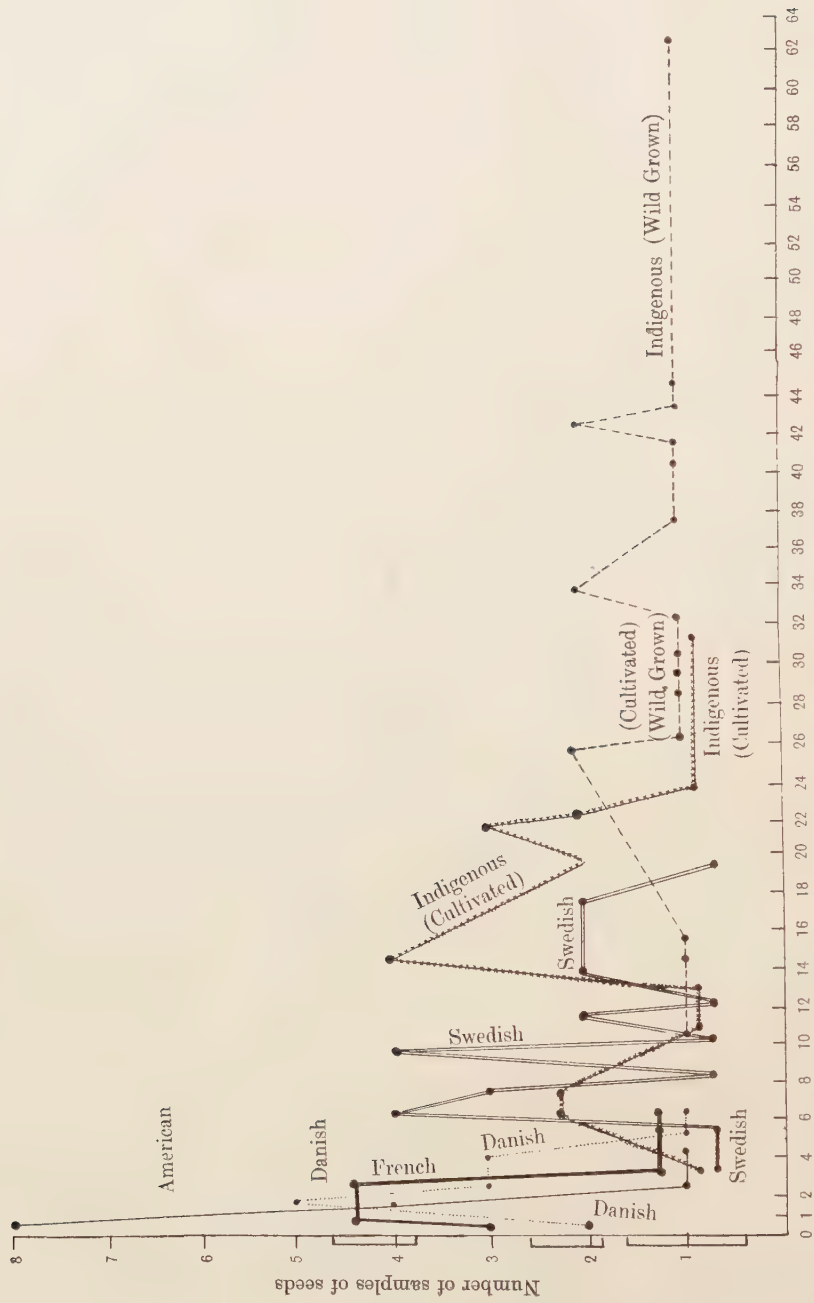


Diagram II. Showing the percentage of damage due to *G. fishericlla* upon series of samples of seeds of different "nationalities" of cocksfoot harvested in 1922.

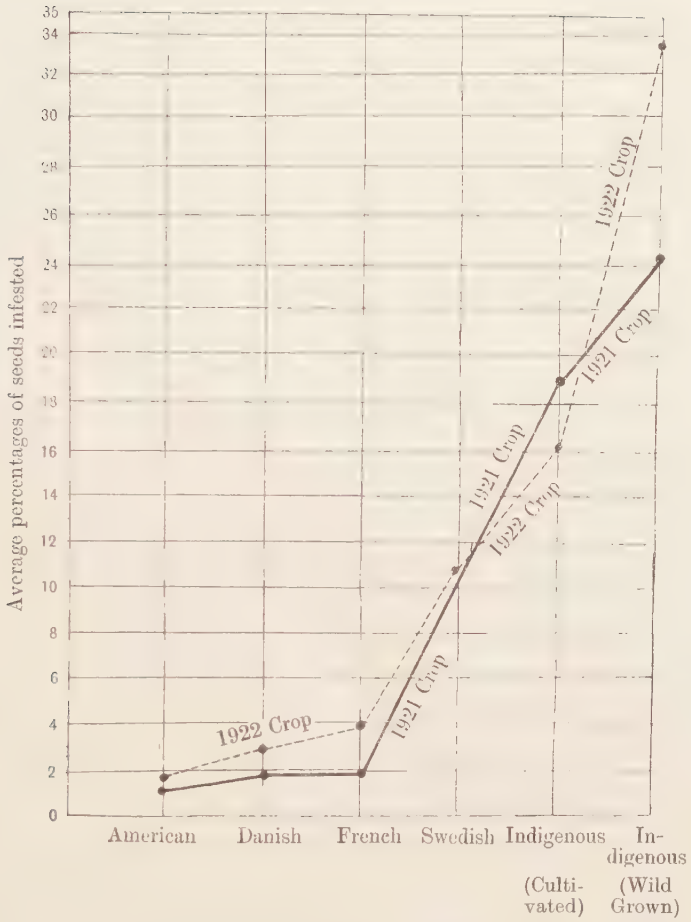


Diagram III. Showing the average percentages of damage due to *G. fischeriella* suffered by the seeds of different "nationalities" of cocksfoot harvested in 1921 and 1922.

physiological differences between different races may repel or attract the female moth at the egg-laying time.

(*a*) *The flowering periods of the plants of the different nationalities in relation to the extent of larval damage caused to the seed.* It has been explained in a previous section that the time of the egg stage of the Cocksfoot Moth and of the flowering stage of the food plants are coincident phenomena. The amount of time taken by the cocksfoot plant to develop its seed is almost the same as that of the incubation period of the egg, viz. about two weeks. Moreover, we know that a large number of eggs are laid during the time when most of the plants are actually in the

flowering condition. It is therefore to be expected that the moth would deposit its eggs on plants the seeds of which would be fully developed at the time of the hatching of the larvae.

The first flowers observed were on June 2nd, and the first moths among the cocksfoot were found on May 30th; the first eggs found upon the seeds were on June 6th. After this date one could rely upon finding eggs by examining about half a dozen panicles of indigenous plants, so I do not think many eggs could be laid before the date named. This was confirmed by some bagging experiments. On May 26th, June 1st and June 7th respectively a couple of indigenous plants, some fifteen panicles in each case, were bagged; in no case was any larva found when bags were removed on June 20th.

As regards the flowering periods of the various nationalities, the facts stand thus for 1922: The French were the first to flower, and their period was between June 2nd and 10th. The Danish and American "nationalities" flowered between June 6th and 13th, and the indigenous between June 8th and 16th. On June 6th almost all the French plants were in flower and also a few American and Danish. On June 8th almost all the French, two-thirds of the American and Danish and a small number of indigenous were flowering. On the 10th the French had passed the maximum flowering stage, the Danish and American were in the period of maximum flowering and the indigenous were approaching the maximum. By the 12th the French had finished flowering, the Danish and American races had passed the maximum and the indigenous were in the maximum flowering stage. On the 14th the Danish and American had finished flowering and the majority of the indigenous had passed the maximum flowering stage. On June 16th four-fifths of the indigenous had finished flowering and many of the foreign were in an advanced stage of seed development. Thus, during the first fortnight in June the plants of all those different races were in flower.

It is true, there are slight differences between the dates of flowering, but if the amount of damage was related to this one would expect that, since the indigenous are most heavily attacked and are the last to flower, the French, which are the first to flower, should show least attack. But they do not, the least attacked being the American. Again, the Danish and the American flower at the same time, so should be attacked equally, which is not the case; and as the time of flowering of the American is only two days earlier than the indigenous, it is difficult to say why the American should be least and the indigenous most attacked of all.

Analysis of the plot showed, moreover, that plants of different

"nationalities," in the same stage of growth and growing side by side, were attacked differentially. For example, among the French, the highest percentage of larval damage was shown to be upon those plants which were growing by the side of the American, and which were in flower at the same time as the latter; the Americans, on the other hand, being almost immune. Of the Danish plants, the ones which suffered most severely were some which stood altogether in one row next to an American row, which was only slightly attacked. The American in general suffered very slight damage, in fact it was not uncommon to find attacked plants growing side by side with immune ones; the indigenous, on the other hand, showed a fairly uniform high extent of damage.

Search for eggs was made upon the individual plants. The first were noticed on June 6th, four days after flowering commenced, on a French plant which was in full flower. Eggs were subsequently found in many other cases on the young seeds when the panicles were in full flower, particularly in indigenous plants. In cases where a large number of eggs were found on indigenous plants which were in the flowering stage, most of the foreign plants which were also flowering at the time and growing close to them, had no eggs deposited upon them. It seems, therefore, that we must seek some selective factor other than times of flowering.

(*b*) *The coloration of panicles at the flowering stage as a possible stimulus for the choice of the plants upon which the eggs are laid.* It was noticed at the flowering stage that certain of the plants, particularly the indigenous, had a kind of steel blue tinge upon the panicles which they retained up to a few days before the harvesting time. The foreign "nationalities" were mostly green while in flower, changing into pale yellow when the seed was fully ripe. But it was by no means rare to find foreign panicles which were also tinged with steel blue and indigenous ones coloured green like the foreign. It appeared possible that this differential coloration might be a factor in affecting the female moth in her choice of plant at the egg-laying time, and indeed, among the indigenous specimens, the steel blue ones invariably suffered heavier damage than others. But, on the other hand, the panicles of the foreign races possessing this steel blue coloration were not more heavily attacked than the green ones. From this one series of observations, it would be rather rash to draw conclusions which might over-estimate the importance of this factor, but I am inclined to think it has some influence in guiding the moth in its choice of food plant.

(*c*) *Selection of food plant by larva.* It might be suggested that the eggs are laid upon the panicles indiscriminately and that upon hatching

the larva migrated to the seeds which it finds most palatable, and that these are the indigenous ones. But, as pointed out above, large numbers of eggs were found on indigenous plants which were in the flowering stage, whereas, most of the foreign plants which were flowering at the same time and growing close to them had no eggs deposited on them. I found no indications of selection by the larva.

(*d*) *Selection of plants by moth, the moth reacting to difference in the physiological strains of the seeds of various races.* It is highly probable that this is the factor mainly responsible for the immunity of the foreign races, and for the comparatively greater damage suffered by the indigenous variety. In a previous section on the habits of adults, it was explained that between the time of the emergence of adults and the egg-laying time, there intervene short periods which are spent by the moths successively on gorse and nettles, during which the cocksfoot plants, as a rule, are scrupulously avoided, although growing by the side of the gorse and nettle. After this stage is passed they commence to visit the cocksfoot plants for the first time after their emergence from the pupa. This shows that the moth is capable of rejecting certain kinds of plants in preference to others. If, therefore, the moth is capable of choosing between two different kinds of plants, is it too much to assume that it is influenced by physiological differences existing between various races of the same kind of plant? Moreover, it is a fact, as noted above, that the eggs are laid upon the seeds of indigenous plants in much greater numbers than upon the foreign ones.

A possible reason for the partiality of the moth towards the indigenous strain is that the latter has been the food plant of the larvae of this species for a much longer time than the foreign strains, which have been introduced into this country only within the last ten years; so that the low percentage of damage caused to foreign seeds may indicate the measure of progress the moth is making in its taste for new strains of seed.

Differential Injury Caused to Indigenous Seeds.

The next problem to consider is that of the higher damage suffered by the wild-growing indigenous cocksfoot plants as compared with the cultivated indigenous plants.

Several factors may contribute to an explanation of this point. We know that the wild plants grow as a rule in hedges and in waste places, where rank growth of vegetation offers a nice shelter to the moth on wet and windy days.

In addition to this, such surroundings are more suitable as a pupation

ground, whereas, a cultivated field, properly manured and frequently cleared of weeds, would be unsuitable as a pupation ground which must therefore be sought away from the plants, entailing a long journey during which the larvae may be exposed to the attacks of birds and parasitic enemies.

Further, the first four days of the life of the adult being spent on gorse and nettle will keep them in the uncultivated areas, and when they migrate to cocksfoot they will naturally settle on the nearest plants.

And when, finally, we recollect that the moth, as we know from its habits, is not a good flier, we seem to have accounted for the greater damage suffered by the wild-growing species.

Loss in weight of cocksfoot seeds due to damage by *Glyphipteryx fischeriella*, Zell.

An attempt was made to estimate the loss by weight of cocksfoot seeds resulting from the ravages of the larva. One thousand healthy seeds of each of the nationalities were weighed; the weight was found to be very similar in each case, the average being 1·2 gm. A thousand attacked seeds of each nationality were then weighed with very similar results in each case, the average weight being 0·72 gm. The loss by weight per thousand seeds was thus found to be 0·48 gm.

To help one to realise what this means in terms of bulk measurement, the loss in pounds per hundredweight has been calculated for each nationality from the above average figures and expressed graphically in Diagram IV.

CONTROL MEASURES.

Out of a few thousand larvae that were reared in the laboratory not a single parasite was observed to emerge. This absence of parasites would appear to be due to the effective protection afforded to the larva owing to its habit of feeding on the kernel inside the seed and generally covering the mouth of the entrance hole while it is feeding by means of a thick roughly spun silken web. While in captivity a small ground beetle which had been brought along with the cocksfoot seed was observed attacking the larvae. But in all likelihood this is not the natural enemy of the larvae. I did not meet with any effective parasite either of the egg or of the larva of this moth[1]. Consequently the method of successfully combating this pest is to be sought in clean husbandry or in some kind of artificial

[1] On one or two occasions I noticed the Common Hedge Sparrow securing this moth on the wing; I had no opportunity to kill the bird and by dissection discover whether this moth is a favourite food.

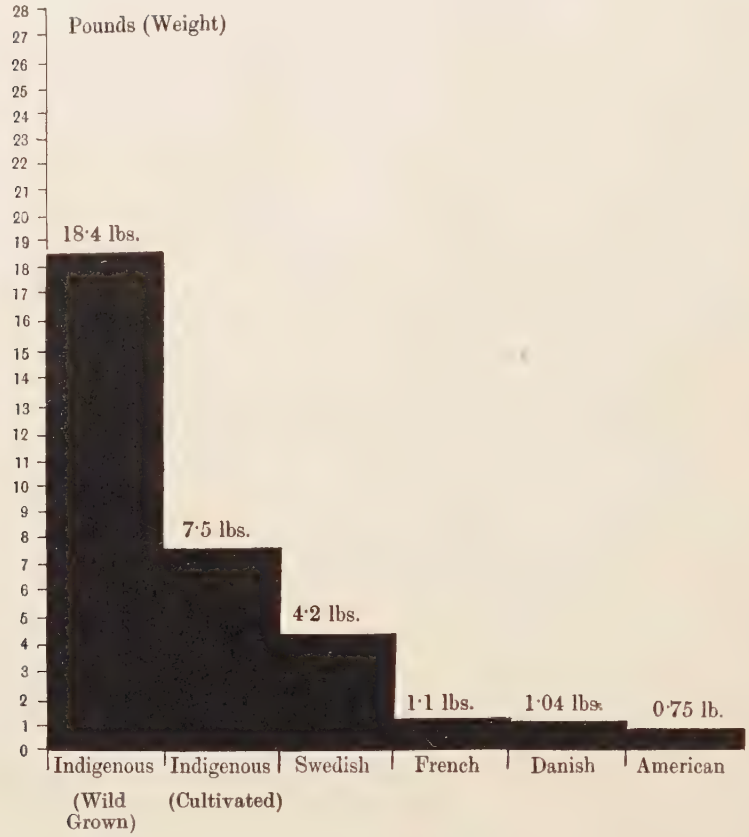


Diagram IV. Showing loss in weight in pound weights per hundredweight (112 lbs.) of the seeds of different "nationalities" of cocksfoot (1922 crop) due to damage by the larvae of *G. fischeriella*.

treatment. From its life-history and from the habits of both the larva and the imago as described above, it appears that clean cultivation will prove to be a valuable method of check.

In the first instance, *all nettles and gorse bushes* in or round about the field should be cut and burnt in the month of March, thus destroying the cocoons together with the larvae of the previous year and also depriving the next generation of their place of shelter during the winter months. As the imagines are known to spend some time prior to the egg-laying period on one or both of these plants, it would be interesting to see whether by the elimination of these it would be possible to control the pest in any appreciable degree.

Secondly, all the weeds and wild plants which offer a handy shelter to the adults on wet or windy days during the period of their activity, namely the first half of June, should be cut and removed from or near the cocksfoot field.

With regard to artificial treatment, one of the easiest and least expensive and at the same time the most effective methods consists in heating the seeds. While drying some seeds in the oven I noticed that many of the larvae which were infesting the seeds were killed by the temperature to which the seeds were subjected. I therefore made several experiments with a view to finding the degree of temperature which, without injuring in any way the germinating power of the seed, would destroy the larvae. Several lots of healthy seeds, together with a number of larvae in each, were kept under different degrees of temperature for varying lengths of time. The following results were obtained:

No. of seeds	No. of larvae	Degree of temperature Centigrade	Time of exposure mins.	Seeds germinated	Percentage of germination
126	28	98°	30	118	93·6
93	23	97°	15	79	84·8
114	37	90°	15	108	97·3
123	27	80°–85°	15	118	95·9
107	19	80°	30	103	96·2
61	13	57°–68°	90	58	95·1
60	13	42°	60	59	98·3

DISTRIBUTION OF *GLYPHIPTERYX FISCHERIELLA*, ZELL. WITHIN AND OUTSIDE THE BRITISH ISLES.

The general distribution of the species in the British Isles extends from the Grampian Range in the North of Scotland to the southernmost counties of England. It occurs all along the eastern coast from Aberdeen down to Yarmouth, and in the west from north Lancashire down to the south-western corner of Pembroke and Devon. In Wales the species has been found practically all over the principality, the actual specimens of attacked seeds having been obtained from the following localities:

Carmarthen (Carmarthenshire), Borth (Cardiganshire), Newport and Pontnewydd (Monmouthshire), Haverfordwest (Pembrokeshire), Caersws (Montgomeryshire), Wrexham (Denbighshire), Brecon and Radnor, Denbighshire, Flintshire and Carnarvonshire.

In Scotland the pest was recorded from Edinburgh, Perth, Dundee and near Aberdeen. It has also been recorded from East Ireland.

It is a striking fact that practically all the samples of panicles which had flowered showed damage.

Outside the British Isles the species has been recorded on the Continent from France and Germany and also from North Africa and Asia Minor.

SUMMARY.

The Cocksfoot Moth (*G. fischeriella*, Zell.) is a small moth (wing expansion $7\frac{1}{2}$–$8\frac{1}{2}$ mm., body dark fuscous, front wings coppery brown, with five silvery streaks along the costal margin.

The moth is diurnal in habits, bright warm weather greatly favours its activities, and on such days from the last week in May till the third week in June it is very common in the field on the nettle and gorse bushes and on flowering stalks of cocksfoot.

The eggs are deposited as a rule singly at the base of a seed in the second and third weeks of June, and hatch in from ten to fifteen days' time.

The larva which does the sole damage is small glassy white when newly hatched, but assumes more and more of green colour as it feeds and grows. When full grown it measures about 6 mm. in length. There are four larval instars altogether.

Immediately after hatching the larva begins to bore into the seed on which it was hatched. The injury takes the form of a small circular hole through which it enters and feeds on the kernel. There is always one entrance hole and only one in each seed which also serves as the exit.

After about four weeks' feeding, when the larva is full grown, it either leaves the food plant and crawls away in search of winter quarters, or remains inside the hollow husk of the seed which previously served as its food. In the former case, it spins a small, oval elongate, dirty white, silken cocoon in which it hibernates during the next nine months prior to actual pupation, while in the latter, instead of constructing a cocoon, it simply seals the entrance hole with a thick silken web.

In captivity, under laboratory conditions, a large number of larvae, by cutting small holes, entered the dry hollow stalks of the cocksfoot plants and constructed cocoons therein.

Pupation takes place in April or early in May, the pupal period extending over three or four weeks.

There is only one generation in a year.

As regards the food plants it was found that indigenous plants of cocksfoot were attacked much more heavily than the foreign "nationalities," as is shown in the following table:

Nationality of seed	Average percentage of damage	
	1921 crop	1922 crop
American	0·71	1·47
Danish	1·29	2·31
Swedish	1·41	2·33
Swedish	No seeds available	9·74
Indigenous (cultivated)	18·46	15·62
Indigenous (natural habitats)	24·21	33·16

A suggestion is made that the moth selects the panicle on which it lays its eggs, finding the indigenous plants more attractive than those of the foreign nationalities. It appears that the selection is made by the moth rather than by the larva.

Samples of seeds obtained from different places in England, Scotland and Wales showed that the pest was generally distributed all over the island; almost all samples examined were found to have been attacked. The larva is so effectively protected within the seed that no internal parasite was found to attack it.

An effective method of obtaining seeds free from this pest is to place them in an airtight chamber, heat up to 60° C., and leave there for twenty-four hours. This temperature kills all the larvae, and, on the other hand, does no harm to the germinating power of the seed.

LITERATURE.

(1) DUPONCHEL, P. A. J. (1838). *Histoire naturelle des Lépidoptères*, XI, 533, pls. 3 and 8, fig. 7.
(2) —— (1844). *Histoire naturelle des Lépidoptères*, Supplement, IV, pl. 77, fig. 3.
(3) ERSCHOFF, N. G. (1892). *Mémoires sur les Lépidoptères.* Rédigés par N. M. Romanoff, VI, 671.
(4) HÜBNER, J. (1826). *Verzeichniss bekannter Schmetterlinge*, p. 421.
(5) MEYRICK, E. (1880). *Proceedings Linnean Society, New South Wales*, V, 247, 248.
(6) —— (1888). *Transactions New Zealand Institute*, XX, 88.
(7) —— (1895). *A Handbook of British Lepidoptera*, p. 704.
(8) —— (1914). "Glyphipterygidae"—Wystman, *Genera Insectorum*, pp. 1–52.
(9) —— (1915). Revision of New Zealand Tineina. *Transactions N. Zealand Institute*, XLVII, 205–244.
(10) —— (1917). Description of N. Zealand Lepidoptera. *Transactions N. Zealand Institute*, L, 125–134.
(11) —— (1917). Description of S. American Microlepidoptera. *Transactions Entomological Society*, London, pp. 1–52.
(12) PRYER, H. J. S. (1877). *Cistula Entomologica*, II, 235.
(13) ROSLERSTAMM, T. (1840). *Abbildung Schmetterlinge*, pl. 82, fig. 3.
(14) SCOPOLI, G. A. (1763). *Entomologia Carniolica.* Insecta Carniolia indigena, etc., n. 658 and 659.
(15) SPULER, A. (1910). *Die Schmetterlinge Europas*, dritte Auflage von E. Hofmann gleichnamigem Werke, II, 299.
(16) STAINTON, H. T. (1859). *Entomologist's Annual*, p. 153.
(17) —— (1867). *British Butterflies and Moths*, p. 40.
(18) ZELLER, P. C. (1839). *Isis*, p. 204.

(*Received July 28th*, 1924.)

A PRELIMINARY NOTE ON THE PERITHECIA OF *NECTRIA GALLIGENA*

By W. A. R. DILLON WESTON, B.A.
Advisory Mycologist, School of Agriculture, Cambridge.

(With 2 Text-figures.)

During March of this year, while investigating the fungal flora of mummied fruits on plum, pear and apple trees, the perithecia of *Nectria galligena* were found present upon a shrivelled apple still adhering to the tree (see Figs. 1 and 2).

About 700 shrivelled apples were examined in the orchard in which this was found and other specimens were obtained. On one practically three-quarters of its surface was studded with the winter stage, the perithecia being fully mature and the asci liberating ascospores. The orchard in question holds a plantation of Worcester Pearmain which suffered a moderately severe attack of canker, young spurs and leaders being girdled.

It is suggested that the formation of perithecia on shrivelled apples may also have taken place in other areas, and that it is a method whereby the fungus over-winters, of which no account has been taken in the past.

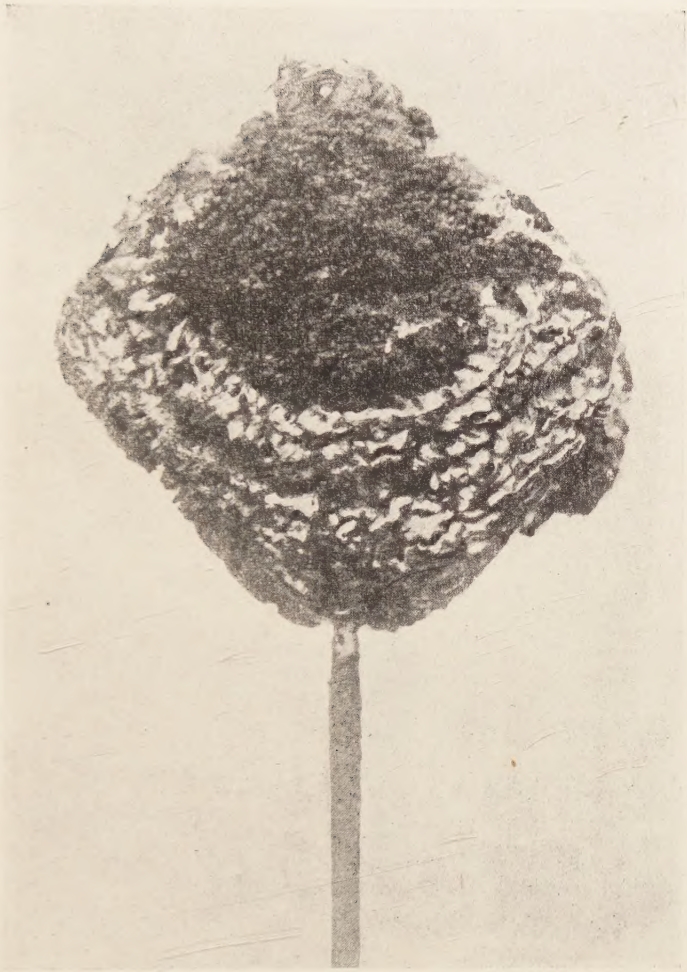


Fig. 1. Shrivelled Worcester Pearmain apple. Surface covered with perithecia of *Nectria galligena*. $\times 4\frac{1}{2}$.

Fig. 2. Shrivelled Worcester Pearmain apple. Surface covered with perithecia of *Nectria galligena*. $\times 4\frac{1}{2}$.

(*Received April 19th*, 1925.)

REVIEW

A General Text Book of Entomology, including the Anatomy, Physiology, Development and Classification of Insects. By A. D. Imms, M.A., D.Sc. Royal 8vo. Pp. 698, with 607 text-figures.

Teachers, students, and research workers have, for some years, felt the need of an up-to-date treatise on Entomology, embodying all the newest discoveries, explaining (and discriminating between) the various theories that have been propounded, and elucidating the complex details of anatomy and physiology of the various Orders of Insects.

Adequately to accomplish all this, within the compass of a single volume, is indeed a difficult task, requiring exceptional knowledge and discrimination. The author sets out (as noted in his preface) with a clear comprehension of these difficulties. How successfully he has overcome them will be apparent to all who study the contents of this volume.

Dr Imms divides his work into three parts: I, Anatomy and Physiology (pp. 1–156). II, Development and Metamorphosis (157–198); III, Classification (198–667). The first two parts, though occupying little more than one-third of the volume, are perhaps the more important. Under these headings the author has marshalled, in the clearest detail, the results of all the best work that has been published to date and has enriched them with his own personal observations. An examination of his "Index of Authors," and of the special references at the end of each section, reveals the vast increase in the serious study of Entomology that has taken place during the present century and the previous decade. This work, hitherto scattered throughout many publications, in various languages, is here brought together for the first time.

After an introductory chapter, dealing with the relationship of Insects with other *Arthropoda*, and describing the general organisation of an Insect, Dr Imms proceeds to deal with the structure of all the parts—external and internal. A careful study of pages 7 and 8 (on Cuticular Appendages) should tend to check a certain looseness of terminology that has been employed by various authors in their description of such structures. Indeed, throughout the present work, we find a particularity of terminology that is most admirable.

Part I deals with the Body Wall, the Head and its appendages, the Limbs and the Wings—with their system of neuration, the Endo-skeleton, the Muscular System, the Nervous System, the Sense Organs (including the physiology of vision), the Sound and Light-producing Organs, the Digestive and Respiratory Systems, the Excretory Organs, the Secretory Glands and the Reproductive System—all of which are described with a clarity of exposition which greatly facilitates the study of these difficult subjects.

Part II is occupied with the Development and Metamorphosis of Insects, under which Embryology receives very full attention.

In the remaining part (III) the author takes us through all the various Orders of Insects, describing the important characters of each Family within those Orders; but wisely refraining from carrying his classification beyond the Families, though space is given to the description of all the more interesting individual types. The *Dermaptera* (Earwigs), sometimes included in the Order *Orthoptera*, appear here as a distinct Order. The often overburdened Order *Neuroptera* is properly restricted to the Alder Flies, Lacewings, Ant Lions, etc.; while the *Plecoptera*, *Isoptera* (Termites or White Ants), *Embioptera*, *Psocoptera*, *Anoplura* (here, for some unexplained reason, rendered "Anopleura," falsely suggesting a derivation from πλευρα—a rib), *Ephemeroptera*, *Odonata*, *Mecoptera* and *Trichoptera* are all assigned to separate Orders, of

equal rank. Similarly, the *Strepsiptera* have been separated from the *Coleoptera*, where they have previously found refuge, and the *Aphaniptera* (Fleas) are no longer classed as degraded *Diptera*.

Of the 607 figures, 183 are original, the remainder being taken from—or adapted from—other authors. These are not, however, the well-known figures that have appeared in text-book after text-book; but have been selected, with great care, from specialists in their particular subjects, and have been excellently reproduced.

Finally, the printing and general "get up" of the volume are most pleasing. The text appears to be very free from errata. The only misprints noticed are: on p. 29, where "artiular sclerites" appears in place of "articular"; and on p. 622 (seventh line from bottom) where "*Contarinia*" has been rendered as "*Contrarina*." On this same page, by the way, is a figure of an antennal joint of *Xylodiplosis praecox*, showing the so-called "circumfili." It is disappointing, however, to find no reference, in the text, explaining these remarkable structures.

E. E. GREEN.